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Preface

This thesis is based on the work carried out at the Technical University of Denmark, Department of Environmental Engineering from October 2015 to October 2018. The research was co-funded by the China Scholarship Council and the Technical University of Denmark, and was performed under the main supervision of Professor Barth F. Smets (DTU Environment).

The thesis is organized in two parts: the first part puts into context the findings of the PhD in an introductive review; the second part consists of the papers listed below. These will be referred to in the text by their paper number written with the Roman numerals **I-IV**.

I. **Su, Q**., Ma, C., Domingo-Félez, C., Kiil, A.S., Thamdrup, B., Jensen, M.M., Smets, B.F., 2017. Low nitrous oxide production through nitrifier-denitrification in intermittent-feed high-rate nitritation reactors. Water Research. 123, 429-438.

II. Blum, J., **Su, Q**.*, Ma, Y., Valverde-Pérez, B., Domingo-Félez, C., Jensen, M., and Smets, B.F., 2018. The pH dependency of N-converting enzymatic processes, pathways and microbes: effect on net-N_2O production. Environmental Microbiology. 20(5), 1623–1640.
*Co-first author

III. **Su, Q**., Domingo-Félez, C., Zhen Z., Blum, J., Jensen, M., and Smets, B.F., N_2O production in an intermittently-fed high-rate nitritation reactor: pH-control is a feasible N_2O mitigation tool. *Submitted to Water Research.*

IV. **Su, Q.**, Domingo-Félez, C., Jensen, M., and Smets, B.F., Abiotic nitrous oxide (N_2O) production shows strong pH dependence, but contributes little to overall N_2O balance in biological nitrogen removal systems. *Submitted to Environmental Science & Technology.*

In addition, the following publication, not concluded in this thesis, was also concluded during the PhD study.

- **Su, Q**., Jensen, M., and Smets, B.F., The effect of pH on N_2O production in an intermittently-fed high-rate nitritation reactor: insights from transcriptomics and isotopic analysis. *Manuscript in preparation.*

This PhD study also contributed to international conferences with the following proceeding and conference papers:

- **Su, Q**., Jensen, M., and Smets, B.F., Low nitrous oxide production in intermittent-feed high performance nitritating reactors. Frontiers International Conference on Wastewater Treatment, 2017, Palermo, Italy. *Flash oral presentation.*

- **Su, Q**., Jensen, M., and Smets, B.F., Low nitrous oxide production in intermittently-fed nitritation reactors. NORDIC wastewater conference, 2017, Aarhus, Denmark. *Flash poster presentation.*

- **Su, Q**., Ma, C., Domingo-Félez, C., Kiil, A.S., Thamdrup, B., Jensen, M.M., Smets, B.F., Low nitrous oxide production through nitrifier-denitrification in intermittent-feed high-rate nitritation reactors. 15th IWA Leading Edge Conference on Water and Wastewater Technologies, 2018, Nanjing, China. *Poster Presentation.*

- **Su, Q**., Domingo-Félez, C., Jensen, M.M., Smets, B.F., N_2O production in an intermittent-feed high-rate nitritation reactor: pH is a feasible N_2O mitigation option. IWA Nutrient Removal and Recovery Conference, 2018, Brisbane, Australia. *Oral Presentation.*

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Thanks to all other colleagues (Ravi, Gorden, Charlotte, others …) at DTU; you all made me enjoy my time while experiencing different cultures. A great thank goes to all my friends Kai, Nannan, Yunjie, Liguan, Sike, Hailin, Liguang, Zhiyong, Wangsheng who have made me not feel alone, cooking, eating, playing and laughing together. Thanks to all brothers and sisters (Sarah, Ruth, Weichu, Fengju, Huizhi, Tengpeng, Peter...) in the Copenhagen Chinese Church, meeting every Sunday to learn GOD’s words, pray and share daily moments of life together.

Finally, I would really like to thank my parents and my brother who have always been there to support me and encourage me. 将赞美和荣耀归于在天上的阿爸父神。

Summary

Massive quantities of inorganic nitrogen (mainly in the form of ammonium (NH_4^+)) in residual waters derived from human activities continue to be released in aquatic ecosystems. Among various physicochemical and biological methods for treating NH_4^+-rich residual waters, biological nitrogen removal (BNR) via nitrification and heterotrophic denitrification process is most widely applied. In recent years, novel processes including nitritation, anammox or a combination of partial nitritation plus anammox (PNA) have been implemented as energy and resource-efficient alternatives of conventional BNR processes. However, emissions of nitrous oxide (N_2O) during the operation of these novel processes may offset the claimed environmental benefits of nitritation or PNA technologies. N_2O is a strong greenhouse with ca. 300 times higher global warming potential than carbon dioxide (CO_2) and contributes to the destruction of stratospheric ozone. Nitrifier nitrification (NN) and nitrifier denitrification (ND) by ammonia oxidizing bacteria (AOB), heterotrophic denitrification (HD) by denitrifying bacteria and several abiotic reactions are identified as pathways of N_2O production. However, the contribution of different pathways of N_2O production and their environmental controls in BNR systems remain to be identified and quantified. Further, a better and quantitative understanding of the mechanisms of N_2O production is warranted, in order to develop operational strategies or system designs that might mitigate N_2O emissions.

This PhD project investigated dynamics, identified pathways, and explored mitigation options for N_2O production in high-rate nitritation reactors. Two lab-scale intermittently-fed sequencing batch reactors were operated towards simultaneous high-rate nitritation and low-rate N_2O emission. The dynamics and constituent pathways of N_2O production were identified and quantified. The effect of pH on N_2O production rates was experimentally examined and the effect of pH on pathway contribution was analyzed using an existing mathematical N_2O process model. A suite of abiotic N_2O production reactions were kinetically determined and the contribution of abiotic reactions to observed N_2O dynamics in the nitritation reactors was estimated. Finally, operational conditions were proposed to minimize N_2O emissions from nitritation reactors.

The reactor biomass was highly enriched in AOB and converted 93 ± 14% of the removed ammonium to nitrite (NO_2^-) at volumetric removal rates of 0.6-0.76 g N/L/d. The dissolved oxygen (DO) set-point (< 0.5 mg O_2/L) com-

bined with intermittent feeding was sufficient to maintain high nitritation rates at 20-26 °C over a period of 710 days. Even at high nitritation efficiencies, net N_2O production was low (ca. 2% of the removed ammonium). *In situ* application of ^{15}N labeled substrates revealed ND as the dominant pathway of N_2O production. Net N_2O production rates transiently increased with a rise in pH (from 7.4 to 7.9) after each pulse feeding, suggesting a potential effect of pH on N_2O production.

To further elucidate the effect of pH on N_2O production, a wide range of pH conditions (pH 6.5-8.5) were imposed on the nitritation reactor. The specific ammonium removal rates and the nitrite accumulation rates remained almost constant at varying pH levels ($p > 0.05$). The specific net N_2O production rates (N_2OR) and the fractional N_2O yield ($\Delta N_2O/\Delta NH_4^+$) increased from pH 6.5 to 8, and decreased slightly at 8.5 ($p < 0.05$). Application of the comprehensive NDHA model suggested ND as the pathway responsible for increased N_2O production at alkaline pH.

Hydroxylamine (NH_2OH) and NO_2^-, intermediates during the nitritation process, can engage in chemical reactions that lead to N_2O formation. The kinetics and stoichiometry of the relevant abiotic reactions were quantified in a series of batch tests across a range of relevant pHs, absence/presence of oxygen, and at different reactant concentrations. The highest N_2O production rates were measured for NH_2OH oxidation by HNO_2, followed by HNO_2 reduction by ferrous iron (Fe^{2+}), NH_2OH oxidation by ferric iron (Fe^{3+}), and finally NH_2OH disproportionation plus oxidation by O_2. Compared to other examined factors, pH had the strongest effect on N_2O formation rates. Acidic pH stimulated N_2O production from the oxidation of NH_2OH by HNO_2 and we could conclude that HNO_2 rather than NO_2^- is the reactant. In departure from previous studies, we estimate that abiotic N_2O production is a minor source (< 3% of total N_2O production) in typical nitritation reactor systems with pH between 6.5 and 8. Only at extremely acidic pH (≤ 5) would the abiotic pathway become significant. In consideration of the effects of pH on both abiotic and biotic N_2O production pathways, circumneutral pH setpoints are suggested to minimize overall N_2O emissions from nitritation systems.

Overall, experimental efforts were implemented to investigate dynamics, pathways and mitigation options for N_2O production in nitritation reactors. This study has identified operational strategies via intermittent feeding and pH control as means to mitigate N_2O emission from nitritation systems.

Dansk sammenfatning

Enorme mængder af uorganisk kvælstof (hovedsageligt i form af ammonium (NH_4^+)) - afledt af menneskelige aktiviteter - udledes fortsat til det akvatiske miljø. Blandt de forskellige fysisk-kemiske og biologiske fremgangsmåder til behandling af spildevand med højt NH_4^+ indhold er biologisk kvælstoffjernelse (BNR) via nitrifikation og heterotrof denitrifikation den proces, der er mest udbredt. I de senere år er nye processer, herunder nitritation, anammox eller en kombination af delvis nitritation og anammox (PNA), blevet implementeret som energi- og ressourceeffektive alternativer for konventionelle BNR processer. Udledninger af lattergas (N_2O) under nitritation eller PNA processen kan imidlertid ophæve de miljømæssige fordele ved disse teknologier. Lattergas er en drivhusgas med et drivhusgaspotentiale ca. 300 gange højere end kuldioxid (CO_2). Således kan selv relativt små mængder af N_2O have stor betydning for den samlede udledning af klimagasser. Yderligere bidrager N_2O til ødelæggelsen af ozonlaget i stratosfæren. Forskellige reaktionsveje kan føre til N_2O produktion: Nitrifikant-nitrifikation (NN) og nitrifikant-denitrifikation (ND) hos de ammoniak-oxiderende bakterier (AOB), heterotrof nitrat/nitrit (NO_3^-/NO_2^-) reduktion til frit kvælstof (N_2) hos de denitrificerende bakterier samt flere abiotiske reaktioner. Der er dog stadig uklart hvilke reaktionsveje og hvor meget de forskellige reaktionsveje bidrager til produktionen af N_2O i BNR systemer samt under hvilke betingelser. En bedre og kvantitativ forståelse af mekanismerne bag N_2O produktionen er endvidere vigtig i udviklingen af styringsstrategier eller system design, der kan nedbringe udledningen af N_2O.

I dette PhD projekt blev N_2O produktionsdynamikken undersøgt, N_2O reaktionsveje identificeret, samt muligheder for at reducere N_2O produktionen udforsket i nitritationsreaktorer med høj aktivitet. To bioreaktorer (sequencing batch reactors, SBRs) med intermitterende medie indløb blev opereret mod målet: Høj nitritation og lav N_2O frigivelse. Dynamikken i N_2O produktionen og de grundlæggende N_2O produktionsveje blev identificeret og kvantificeret. Effekten af pH på N_2O produktionsrater blev eksperimentelt undersøgt og effekten af pH på bidraget af de forskellige N_2O produktionsveje blev analyseret ved at anvende en tidligere udviklet matematisk N_2O procesmodel. En række abiotiske N_2O produktionsveje blev undersøgt via reaktionskinetik og bidraget af de abiotiske reaktioner på den observerede N_2O dynamik i nitritation reaktorerne blev anslået. Forskellige styringsstrategier blev foreslået til at minimere frigivelsen af N_2O fra nitritation reaktorerne.

Biomassen i reaktorerne bestod mest af AOB og omdannede 93 ± 14% af den fjernede ammonium til nitrit (NO_2^-) med rater på ca. 0.6-0.76 g N/L/d. Den indstillede værdi på opløst ilt (DO) (< 0.5 mg O_2/L) kombineret med intermitterende medie indløb var med til at opretholde høje nitritationsrater ved en temperatur på 20-26 °C og over en periode på 710 dage. Selv ved høje nitritationsrater, var netto produktionen af N_2O lav (ca. 2% af al ammonium fjernet). Anvendelsen af ^{15}N-mærkede kvælstofforbindelser afslørede ND som den dominerende N_2O produktionsproces. Netto produktionsrater af N_2O steg midlertidigt samtidigt med en stigning i pH (fra 7.4 til 7.9) efter hvert medietilsæt, hvilket antyder at pH har en potentiel virkning på N_2O produktionen.

For yderligere at belyse virkningen af pH på N_2O produktionen, blev forskellige pH værdier (pH 6.5-8.5) testet på nitritation reaktoren. De specifikke ammonium oxidationsrater og nitrit akkumuleringsrater forblev næsten konstant selvom pH værdier varierede ($p > 0.05$). Specifikke netto N_2O produktionsrater (N_2OR) og fraktionen af N_2O ($\Delta N_2O/\Delta NH_4^+$) steg ved pH ændring fra 6.5 til 8, og faldt en smule ved pH 8.5 ($p < 0.05$). Anvendelse af NDHA modellen for N_2O dynamik indikerede at ND kan være den ansvarlige reaktionsvej for den forøgede N_2O produktion ved alkalisk pH.

Hydroxylamin (NH_2OH) og NO_2^-, der begge er mellemprodukter i nitritationen, kan indgå i kemiske reaktioner, som fører til dannelse af N_2O. Kinetikken og støkiometrien af de relevante abiotiske reaktioner blev kvantificeret i en serie af batch forsøg med varierende pH værdier, med og uden ilt, og med forskellige reaktant koncentrationer. De højeste N_2O produktionsrater blev målt for NH_2OH oxidation med hydrogen dixoxo nitrat (HNO_2), efterfulgt af HNO_2 reduktion via reduceret jern (Fe^{2+}), NH_2OH oxidation via oxideret Fe og til sidst NH_2OH disproportionering plus oxidation via O_2. Sammenlignet med de andre undersøgte faktorer, havde pH den stærkeste effekt på N_2O produktionsrater. Lav pH stimulerede N_2O produktionen under oxidationen af NH_2OH med HNO_2, og vi kunne konkludere, at HNO_2 i stedet for NO_2^- er reaktanten. Modsat tidligere undersøgelser anslås, at abiotisk N_2O produktion er en mindre kilde til N_2O produktionen (< 3% af den samlede N_2O produktion) i typiske nitritation reaktorsystemer, hvor pH ligger imellem 6.5 og 8. Kun ved ekstremt lav pH (≤ 5) ville den abiotiske reaktionsvej være betydningsfuld. Ved at sammenholde virkningerne af pH på både abiotiske og biologisk N_2O produktionsprocesser, er fastholdelse af neutral pH foreslået som et redskab til minimere den samlede N_2O frigivelse fra nitritation systemer.

Samlet set blev dynamikken og reaktionsveje for N_2O produktion samt muligheder for nedbringelse af N_2O produktion i nitritationsreaktorer undersøgt igennem forskellige eksperimentelle forsøg. Resultaterne viste at intermitterende medie tilløb og pH-kontrol er effektive strategier til reducere frigivelsen af N_2O fra nitritation systemer.

Table of contents

Abbreviations

AMO	ammonia monooxygenase
ALR	ammonium loading rate
AnAOB	ammonia oxidizing bacteria
AOB	ammonia oxidizing bacteria
ARR	ammonium removal rate
BNR	biological nitrogen removal
CO_2	carbon dioxide
COD	chemical oxygen demand
cyt	cytochrome
diH_2O	deionized water
DO	dissolved oxygen
EPS	extracellular polymeric substances
FA/NH_3	free ammonia
FNA/HNO_2	free nitrous acid
GHG	important greenhouse gas
HAO	hydroxylamine dehydrogenase
HD	heterotrophic denitrifier/denitrification
HDH	hydrazine dehydrogenase
HNO	nitroxyl
HRT	hydraulic retention time
HZS	hydrazine synthase
k	rate constant
$K_{AOB.NH3}$	NH_3 affinity constant
$K_{AOB.I.NH3}$	NH_3 inhibition affinity constant
$K_{AOB.HNO2.ND}$	HNO_2 affinity constant
$K_{AOB.\ I.HNO2.\ ND}$	HNO_2 inhibition constant
$K_{AOB.NH2OH.ND}$	NH_2OH affinity constant during NO reduction
$K_{AOB.O2.I}$	O_2 inhibition constant
$k_L a_{N_2O}$	mass transfer coefficient
LCA	life cycle assessment
LCC	life cycle costing
LCCA	life cycle and cost analysis
MLVSS	mixed liquor volatile suspended solid
N	nitrogen

N_2	molecular nitrogen
N_2H_4	hydrazine
N_2O	nitrous oxide
N_2OR/ r_{N2O}	N_2O production rate
NAR	nitrate reductase
ND	nitrifier denitrification
NDHA	nitrifier nitrification, nitrifier denitrification, heterotrophic denitrification and abiotic reaction
NH_2OH	hydroxylamine
NH_4^+	ammonium
NhAR	hydroxylamine accumulation rate
NiAR	nitrite accumulation rate
NIR	nitrite reductase
NN	nitrifier nitrification
NO	nitric oxide
NO_2^-	nitrite
NO_3^-	nitrate
NOB	nitrite oxidizing bacteria
NOR	nitric oxide reductase
NOS	nitrous oxide reductase
NXR	nitrite oxidoreductase
O_2	oxygen
PNA	partial nitritation-anammox
PSD	particle size distribution
r_{Fe2+}	Fe^{2+} depletion rate
r_{NH2OH}	NH_2OH depletion rate
SBR	sequencing batch reactor
SRT	sludge retention time
VER	volumetric exchange ratio
WRRF	water resources recovery facility
WWTPs	wastewater treatment plants
η_{NIR}	anoxic reduction factor for NO_2^- reduction
$\Delta N_2O/\Delta NH_4^+$	N_2O produced per NH_4^+ removed
$\mu_{AOB.AMO}$	maximum AMO-mediated reaction rate

1 Introduction

1.1 Biological nitrogen removal

As one of critical chemical elements in the Earth, nitrogen (N) is essential to many key biomolecules (Seinfeld and Pandis, 2006). The major forms of N are molecular nitrogen (N_2) and a small proportion in biologically available inorganic N, such as ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-). Over the past decades, more than doubled amount of inorganic N derived from human activities was released into aquatic ecosystems, leading to serious environmental threats (Vitousek et al., 1997). The excess NH_4^+ in waters that activates nitrification process causes significant oxygen depletion, while high concentrations of NO_2^- and NO_3^- are toxic for oxygen-respiring animals. Hence, nitrogen (mainly in the form of NH_4^+) in wastewater has become particular focus of treatment processes. In the following, novel biological nitrogen removal (BNR) processes are summarized; relevant microorganisms in BNR systems are introduced; strategies to achieve high-rate nitritation are proposed.

1.1.1 Processes

NH_4^+ can be removed from wastewater streams by a variety of physicochemical and biological processes. Compared to the physicochemical processes, BNR processes are more efficient and economic (EPA, 1993). Among available BNR technologies, traditional nitrification-denitrification process is the most applied, where NH_4^+ is converted to NO_3^- in a two-steps process under oxic conditions and then NO_3^- is subsequently reduced to N_2 under anoxic conditions. However, this conventional process is costly due to excess aeration and exogenous addition of organic carbon source (Van Loosdrecht and Jetten, 1998). To overcome the existing limitation of traditional process, several novel technologies, including nitritation, anaerobic ammonium oxidation (anammox), and the combined systems of partial nitritation–anammox (PNA) and nitritation-denitritation have been developed.

Nitritation

Nitritation involves the oxidation of NH_4^+ to NO_2^- by ammonia oxidizing bacteria (AOB) under oxic conditions:

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O \qquad \text{(Eq. 1)}$$

This process can be operated in a simple continuous aerated reactor and is ideally suitable to remove NH_4^+-rich wastewater (> 0.5 g N/L), such as rejection-water and landfill leachate (Hellinga et al., 1998; Jetten et al., 1997). As the oxidation is stopped at the nitrite stage, nitritation greatly reduces the expense of aeration and saves energy (Fig.1.1). However, it often remains difficult to maintain stable nitritation performance over the long-term period, especially in large-scale operations (this is further discussed in 1.1.3).

Anammox

During anammox process, NH_4^+ together with NO_2^- are converted to N_2 by anaerobic ammonia oxidizing bacteria (AnAOB) under anoxic conditions (Strous, 2000):

$$NH_4^+ + 1.3\ NO_2^- \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 2\ H_2O \qquad \text{(Eq. 2)}$$

The process can be carried out in fixed or fluidized bed reactors and has a good potential to remove NH_4^+ from sludge digestion effluent (Strous et al., 1997). This is a promising alternative for nitrogen removal as no carbon addition required and little sludge produced (Jetten et al., 1997; van de Graaf et al., 1996). However, the low biomass yield of AnAOB due to their slow growth rate also calls for long sludge retention and start-up time in order to obtain a sufficient biomass concentration.

PNA

The PNA process is defined by a 50% conversion of influent NH_4^+ to NO_2^- by AOB, while the remaining NH_4^+ is oxidized with NO_2^- to produce N_2 in the anammox process:

$$NH_4^+ + 0.85\ O_2 \rightarrow 0.435\ N_2 + 0.13\ NO_3^- + 1.3\ H_2O + 1.4\ H^+ \qquad \text{(Eq. 3)}$$

This process can be achieved either in one- or two-stage systems. Although the two-stage process requires higher investment costs related to construction, this configuration allows for coordination and optimization of the individual conversion stages (Desloover et al., 2011). The PNA process is particularly suitable for industrial wastewaters with high NH_4^+ concentrations and a deficiency in organic carbon (Khin and Annachhatre, 2004). The significant advantages of PNA process include lower operational costs due to lower aeration needs (up to 60% compared traditional BNR), lower carbon footprint emission without external carbon addition, lower bioreactor volume, lower excess sludge production and higher nitrogen removal efficiency (Kartal et al., 2010; Siegrist et al., 2008). Nevertheless, wide implementation of this

process is still challenge because of high optimal temperature (30-35 °C), slow bacteria specific growth rate and high sensitivity to condition changes.

Nitritation-denitritation

This process implies the oxidation of NH_4^+ to NO_2^- (nitritation) and its denitrification to N_2 (Fux et al., 2006; Ruiz et al., 2005). In contrast to the conventional nitrification-denitrification via nitrate, the nitritation-denitritation requires 25% less aeration energy and 40% less external carbon addition (Fig.1.1). In order to perform this process, stable nitritation performance should be maintained and denitrifiers should adapt to NO_2^-, which is toxic at low concentrations

Nowadays, driven by sustainability challenges e.g. climate change and resource depletion, the potential economic and environmental gains of both conventional and novel BNR technologies must be evaluated not only at process level but also for the whole water resources recovery facility (WRRF) (Agrawal et al., 2018). The wastewater treatment industry is undergoing a paradigm shift in technology development from wastewater treatment to resource recovery (Lin et al., 2016). A life cycle and cost analysis (LCCA) is suggested to assess the sustainability of mainstream PNA applications, where costs (via life cycle costing, LCC) and environmental impact (via life cycle assessment, LCA) are simultaneously included in process optimization (Agrawal et al., 2018) (Fig.1.1).

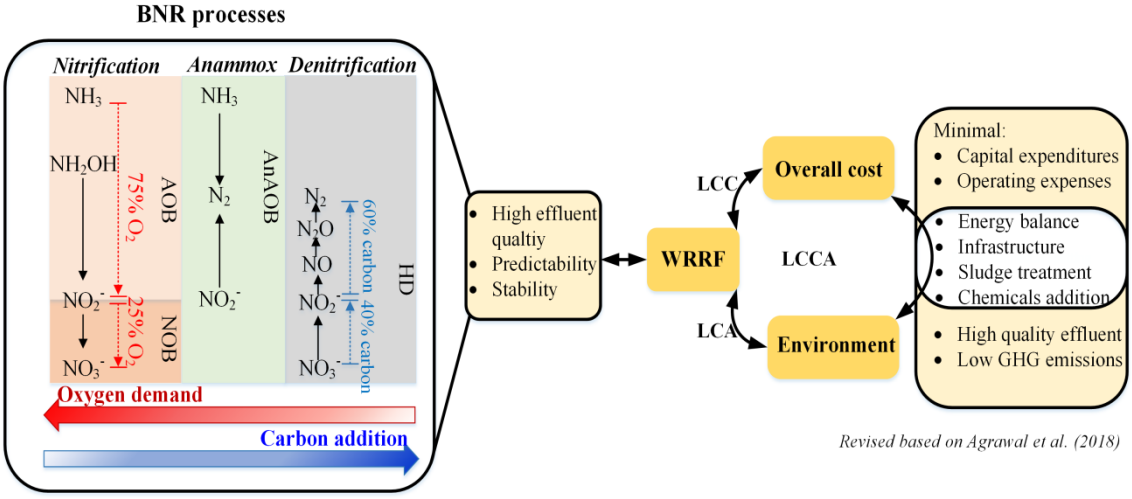

Fig.1.1 Schematic illustration and life cycle and cost analysis (LCCA) of the biological nitrogen removal (BNR) processes. The integration of life cycle costing (LCC) and life cycle assessment (LCA) in a LCCA superstructure is to evaluate the sustainability of BNR processes. WRRF is the abbreviation of water resources recovery facility.

1.1.2 Microorganisms

The microbial community in wastewater treatment plants (WWTPs) is complex and commonly comprised of bacteria and a small fraction of archaea (Tomaszewski et al., 2017). Microorganisms in WWTPs are capable of nitrogen removal, sulfate reduction, iron reduction, phosphate and glycogen accumulation. In BNR systems, the key microbial actors of N conversion are: AOB, nitrite oxidizing bacteria (NOB), heterotrophic denitrifying bacteria and AnAOB. For traditional nitrification-denitrification, AOB together with NOB are responsible for complete nitrification (i.e. NH_4^+ to NO_3^-), while heterotrophic denitrifiers convert produced NO_3^- to N_2 via denitrification process. For individual or combined nitritation and anammox process, only AOB and AnAOB are required while NOB are undesired because they compete with AOB for oxygen (O_2) and with AnAOB for NO_2^-. The effective selection of AOB over NOB is vital to maintain stable nitritation performance. Despite of limited organic carbon in nitritation and anammox systems, denitrifiers could still survive dependent on hydrolyzed products originated from biomass decay and play a role in N conversions as well.

Ammonia oxidizing bacteria

AOB oxidize free ammonia (NH_3, FA) to NO_2^- in a two-step process. The NH_3 is firstly oxidized to hydroxylamine (NH_2OH) catalyzed by ammonia monooxygenase (AMO), where two electrons are required (Kostera et al., 2010) (Eq. 4). During the second step, the subsequent oxidation of NH_2OH to NO_2^- is catalyzed by hydroxylamine dehydrogenase (HAO) (Eq. 5). This step releases four electrons, two for sustaining NH_3 oxidation and the remaining two for energy production. More recently, nitric oxide (NO) has been recognized as another obligate intermediate produced by HAO (Caranto and Lancaster, 2017). Caranto and Lancaster (2017) suggested that NH_2OH is oxidized by HAO to NO using three electrons under both anoxic and oxic conditions. Nitrite (NO_2^-) is thereafter produced from a non-enzymatic oxidation of NO by O_2 under oxic conditions.

In addition, AOB can reduce NO_2^- to nitrous oxide (N_2O) catalyzed by nitrite reductase (NIR) and nitric oxide reductase (NOR) through the nitrifier denitrification (ND) pathway under low oxygen tensions (Wrage et al., 2001) (Eq. 6-7).

$$NH_3 + O_2 + 2\,H^+ + 2\,e^- \xrightarrow{\text{AMO}} NH_2OH + H_2O \qquad \text{(Eq. 4)}$$

$$NH_2OH + H_2O \xrightarrow{HAO} HNO_2 + 4\,H^+ + 4\,e^- \quad \text{(Eq. 5)}$$

$$NO_2^- + 2\,H^+ + e^- \xrightarrow{NIR} NO + H_2O \quad \text{(Eq. 6)}$$

$$2\,NO + 2\,H^+ + e^- \xrightarrow{NOR} N_2O + H_2O \quad \text{(Eq. 7)}$$

AOB found in WWTPs are mainly affiliated to *Nitrosomonas*, *Nitrosospira*, *Nitrosovibrio* and *Nitrosococcus* genera, with *Nitrosomonas* and *Nitrosospira* as the most dominant AOB populations (Kowalchuk and Stephen, 2001). In an ecological context, *Nitrosomonas* and *Nitrosospira* genera are known as r- and k-strategists, respectively, indicating a higher specific growth rate and a lower substrate affinity of *Nitrosomonas* than *Nitrosospira* (Terada et al., 2013).

Nitrite oxidizing bacteria

NOB are aerobic chemolithoautotrophs that oxidize NO_2^- to NO_3^- catalyzed by the enzyme nitrite oxidoreductase (NXR) (Eq. 8). Two electrons are released during NO_2^- oxidation and transferred to O_2 via a respiratory chain for water generation (Hiatt and Grady, 2008) (Eq. 9).

$$NO_2^- + H_2O \overset{NXR}{\longleftrightarrow} NO_3^- + 2\,H^+ + 2\,e^- \quad \text{(Eq. 8)}$$

$$2\,H^+ + 2\,e^- + 0.5\,O_2 \rightarrow H_2O \quad \text{(Eq. 9)}$$

NOB have been found in several genera distributed among different phylogenetic lineages of bacteria, i.e. *Nitrobacter*, *Nitricoccus*, *Nitrispina*, *Nitrispira* and *Nitrotoga*. Species of the genus *Nitrobacter* are the best characterized NOB, though *Nitrospira* species are often the numerically dominant NOB in WWTPs and constitutes the most diverse group of known NOB (Daims et al., 2001). For growth kinetics, *Nitrobacter spp*. are recognized as r-strategists, being generally outcompeted by *Nitrospira spp*. (k-strategists) at low NO_2^- concentrations. For individual or combined nitritation and anammox process, NOB are undesired because they compete with AOB for O_2 and with AnAOB for NO_2^-, decreasing process performance. Therefore, an efficient suppression of NOB is crucial for stable nitritation and nitritation-anammox performance. One of the feasible options is to manipulate operational parameters, such as low dissolved oxygen (DO) and high NH_4^+ loadings, that are favorable for AOB over NOB (Blackburne et al., 2008; Vadivelu et al., 2007). To date, it is still a challenge to completely eliminate NOB from microbial communities (Domingo-Félez et al., 2014).

Heterotrophic denitrifiers

Denitrifying bacteria are commonly heterotrophs that stepwise reduce NO_3^- to N_2 via several free intermediates (NO_2^-, NO and N_2O) under anoxic or suboxic conditions (Eq. 10). The four reductive steps are enzymatically catalyzed by nitrate reductase (NAR), NIR, NOR and nitrous oxide reductase (NOS), respectively (Zumft, 1997). Heterotrophic denitrifiers identified in WWTPs are often closely affiliated with *Proteobacteria* and *Bacteroidetes* (Lu et al., 2014).

$$NO_3^- \xrightarrow{NAR} NO_2^- \xrightarrow{NIR} NO \xrightarrow{NOR} N_2O \xrightarrow{NOS} N_2 \qquad \text{(Eq. 10)}$$

In nitritation or PNA systems, even without any addition of organic carbon, denitrifiers could still survive dependent on the presence of extracellular polymeric substances (EPS) and decay products originated from biomass, even in autotrophic systems (**Paper I & III**). Even though they do not directly contribute to the NH_4^+ removal, denitrifiers offer potential routes to produce and also consume N_2O. Denitrifiers were found to produce N_2O as an intermediate under oxygen inhibition, low C/N and high NO_2^- concentrations (Domingo-Félez et al., 2017b; Schulthess et al., 1995; Wunderlin et al., 2012). Denitrifying activity could also significantly affect the N_2O production via ND pathway by AOB as these two denitrification processes have similar affinities for nitrogen substrate and oxygen (Shen et al., 2015). Additionally, denitrifiers can be underlying N_2O-sinks because they possess the genetic potential (i.e. *nosZ* gene, which encodes NOS) to reduce N_2O to N_2. So far, two different bacterial clades (i.e. clade I and II) have been defined in *nosZ* carrying organisms. However, not all denitrifiers possess the gene (Graf et al., 2014). The reasons for incomplete denitrification are debatable and niche differentiation may play a role (Graf et al., 2014; Jones and Hallin, 2010).

Anaerobic ammonia oxidizing bacteria

AnAOB perform the oxidation of NH_4^+ with NO_2^- to N_2 via NO and hydrazine (N_2H_4) (Strous et al., 1998). These sequential reactions are catalyzed by the enzymes of NIR, hydrazine synthase (HZS) and hydrazine dehydrogenase (HDH) (Kartal et al., 2011) (Eq. 11-13). AnAOB possess an intracytoplasmic compartment, called the "anammoxosome", in which all three enzymes for catabolism are located (Niftrik et al., 2004). AnAOB in WWTPs mainly affiliate with the genera *Kuenia*, *Brocadia*, *Anammoxoglobus* and *Jettenia* (Kartal et al., 2013).

$$NO_2^- + 2\,H^+ + e^- \xrightarrow{NIR} NO + H_2O \quad \text{(Eq. 11)}$$

$$NH_4^+ + NO + 2\,H^+ + 3\,e^- \xrightarrow{HZS} N_2H_4 + H_2O \quad \text{(Eq. 12)}$$

$$N_2H_4 \xrightarrow{HDH} N_2 + 4\,H^+ + 4\,e^- \quad \text{(Eq. 13)}$$

AnAOB are slow-growing microorganisms, which bring both advantages (reduced excess sludge production) and disadvantages (increased sludge retention and start-up time) for the application of the anammox technology (Kartal et al., 2010; Siegrist et al., 2008). Although N_2O is not believed to be produced or consumed by the AnAOB themselves (Kartal et al., 2007), intermediates (e.g. NO_2^- and NO) produced during the anammox process may affect the N_2O production by AOB or denitrifiers.

1.1.3 Strategies to achieve high-rate nitritation

In order to perform novel processes of nitritation and PNA, high and stable nitritation performance must be maintained. The key strategy to achieve nitritation is selective enrichment of AOB over NOB, according to their different growth characteristics mentioned above. Various parameters such as DO, FA, free nitrous acid (FNA), temperature and feeding strategy have been developed to enrich AOB over NOB (Blackburne et al., 2008; Hellinga et al., 1998; Liu and Wang, 2014; Vadivelu et al., 2007; Yang et al., 2013).

DO

Oxygen limitation is a critical factor to achieve and maintain high nitritation performance. AOB are postulated to outcompete NOB at low DO concentrations due to the higher oxygen affinity of AOB than NOB (Blackburne et al., 2008; Wiesmann, 1994). However, there are some contradictory findings that nitritation cannot be achieved in some low-DO reactors (Liu and Wang, 2013). One of potential explanation is that oxygen affinity values may vary within the functional groups and even at strain levels, such as seemingly higher oxygen affinity values of *Nitrobacter* (0.17-8.2 mg/L) than *Nitrosomonas* (0.033-1.21 mg/L) reported in literature (Mutlu, 2015).

FA and FNA

High concentrations of FA and FNA are known to suppress both AOB and NOB, but each clade shows different sensitivities (Anthonisen et al., 1976). NOB are reported to be more sensitive towards FA and FNA than AOB: NOB were totally inhibited at a FA concentration of 0.1-1 or 6 mg NH_3-N/L, and a

FNA level of 0.02-0.2 mg HNO_2-N/L, while the inhibitory concentrations of FA and FNA for AOB were 10-150 mg NH_3-N/L and 0.4 mg HNO_2-N/L, respectively (Anthonisen et al., 1976; Vadivelu et al., 2007, 2006).

Temperature

Temperature is another parameter affecting the relative competitiveness of AOB over NOB. AOB are generally assumed to grow faster than NOB at evaluated temperature (> 20 ^oC) (Bougard et al., 2006; Hellinga et al., 1998).

Feeding strategy

Intermittent feeding has been reported to enhance nitritation rate and NO_2^- accumulation in A/O sequencing batch reactors (SBRs) (Lemaire et al., 2008; Yang et al., 2007). Compared to the instantaneous feeding mode, intermittent feeding forces lower NH_4^+ loading and shorter - but more frequent - feast-famine conditions (Wang et al., 2012).

Despite of feasible strategies mentioned above, it still remains a challenge to establish and also maintain stable nitritation over the long-term. Because the microorganisms could manifest different affinities for substrate after being exposed a certain limiting condition for long periods. For instance, AOB and NOB have been shown to acclimate to limiting environmental conditions of low DO (by enhancing heme protein expression) (Arnaldos et al., 2013) or high concentrations of FA and FNA (Turk and Mavinic, 1989, 1986). However, specific operational conditions in nitritation system, such as low DO, high NH_4^+ and NO_2^- may promote accumulation and emission of the greenhouse gas N_2O (Kampschreur et al., 2008; Kim et al., 2010; Mampaey et al., 2016; Peng et al., 2015a, 2014; Tallec et al., 2006). In comparison to conventional nitrification-denitrification processes, nitritation reactors were reported to produce more N_2O (up to 17% of the NH_4^+ removed) (**Paper I)**. Hence, the increased N_2O offsets the environmental benefits of nitritation or PNA systems.

1.2 N_2O production during biological nitrogen removal

Emissions of greenhouse gasses (GHG) to the atmosphere are of concern. N_2O is a greenhouse gas with large radiative forcing properties (its global warming potential is 300 times higher than carbon dioxide (CO_2)), and has

stratospheric ozone depletion potential (IPCC, 2013; Strokal and Kroeze, 2014). The global N_2O emissions can account for 6-8% of the total anthropogenic GHG emissions expressed in CO_2 equivalents (Fig.1.2) (IPCC, 2013). Moreover, 3.2% of the anthropogenic N_2O emissions are linked to sewage treatment. Taking into account N_2O emissions from manure, landfill leacheates and industrial nitrogenous effluents, this number increases to 10.2% (Fig.1.2) (Desloover et al., 2012; IPCC, 2013). At the WWTP level, N_2O emissions can be very important: a 1% rise of N_2O emission factor increases the carbon footprint of the whole plant by 50%, reaching up to 80% of operational CO_2 footprint (Desloover et al., 2012; Monteith et al., 2005). Nitritation process in BNR system is generally recognized as the main contributor for N_2O production, due to the specific operational conditions applied (e.g. low DO and high NO_2^-) (Desloover et al., 2012). The N_2O emissions from both lab-scale and full-scale PN reactors were reported to reach up to 17% of the NH_4^+ removed (Desloover et al., 2011; Gao et al., 2016; Kong et al., 2013; Lv et al., 2016; Mampaey et al., 2016). To avoid environmental benefits of nitritation/PNA process being offset by N_2O emissions, a better quantitative understanding of the mechanisms for N_2O production is crucial to develop novel strategies or new designs to mitigate N_2O (**Paper I**). In the following section, the key metabolic pathways involved in N_2O production in BNR systems, factors influencing N_2O emissions and proposed mitigation strategies are summarized.

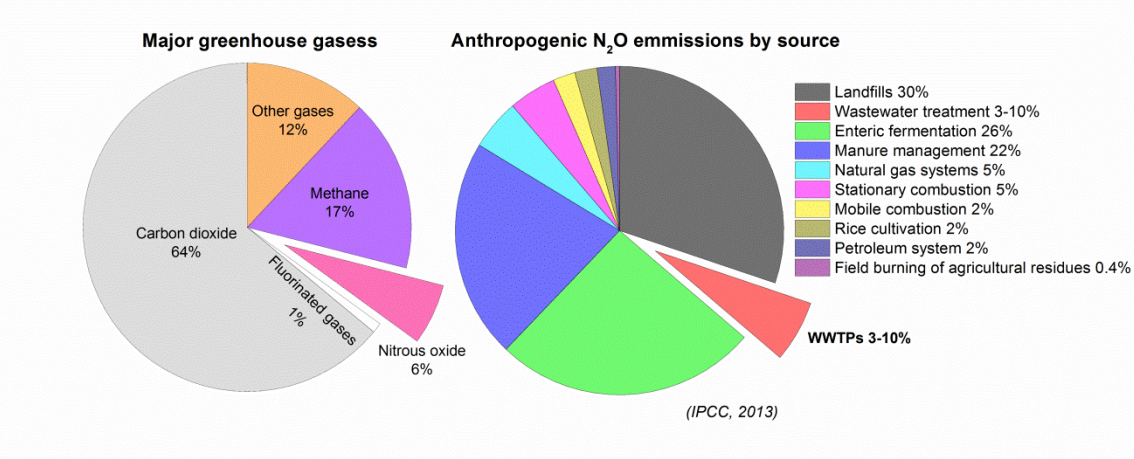

Fig.1.2 Anthropogenic greenhouse gases and N_2O emissions sources (IPCC, 2013).

1.2.1 N_2O production and consumption pathways

N_2O can be produced during biotic or abiotic N conversions in BNR systems. The main biological pathways involved in N_2O production are nitrifier nitrifi-

cation (NN), ND and heterotrophic denitrification (HD) (Blum et al., 2018; Schreiber et al., 2012), which are schematically depicted in Fig.1.3. Besides, the reactive intermediates (e.g. NH_2OH and NO_2^-) produced during nitritation may engage in chemical reactions, leading to N_2O production, especially in the presence of trace metals (e.g. Fe^{2+}/ Fe^{3+}) (Schreiber et al., 2012).

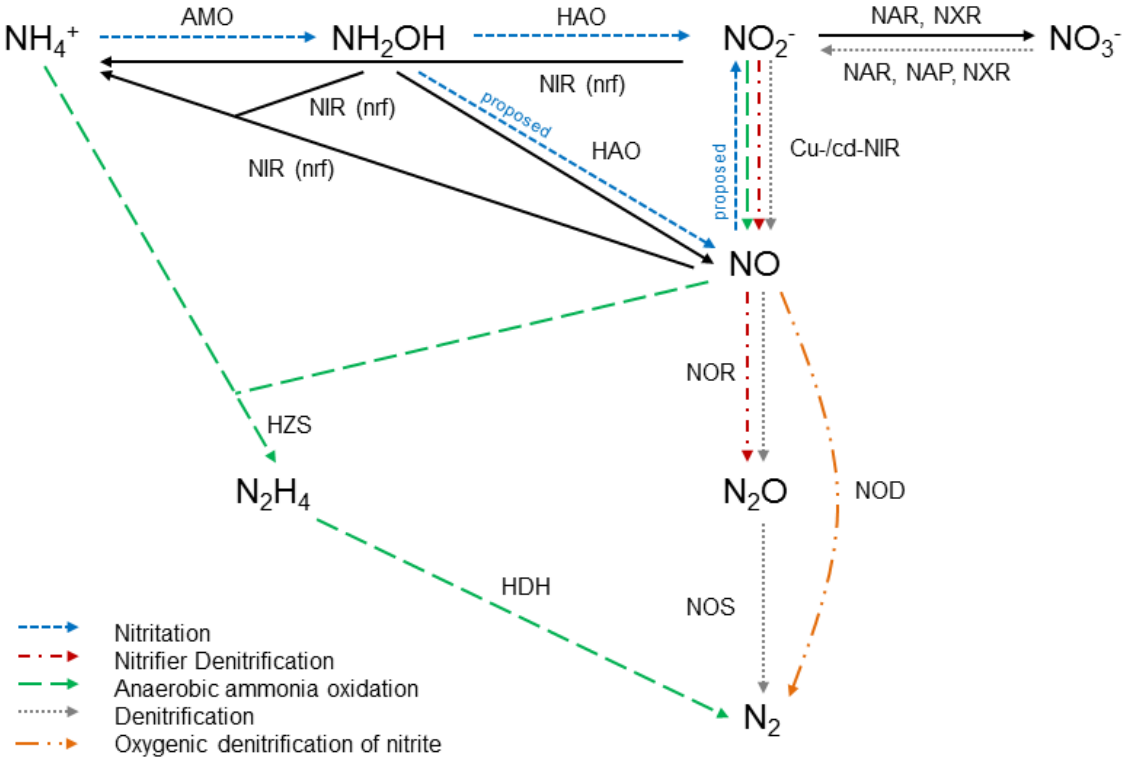

Fig.1.3 Web of nitrogen conversion reactions. Enzymes involved in catalysis are ammonia monooxygenase (AMO), hydroxylamine dehydrogenase (HAO), nitrate reductase (NAR), periplasmic nitrate reductase (NAP), nitrite oxidoreductase (NXR), nitrite reductase (NIR), nitric oxide reductase (NOR), nitrous oxide reductase (NOS), hydrazine synthase (HZS), hydrazine dehydrogenase (HDH) and nitric oxide dismutase (NOD) (Figure from Paper II).

Nitrifier nitrification

N_2O is a byproduct during incomplete oxidation of NH_2OH by HAO in AOB, through intermediates of NO or nitroxyl (HNO) that can be further converted to N_2O biologically or chemically (Caranto and Lancaster, 2017; Law et al., 2012; Poughon et al., 2001; Tallec et al., 2006) (Fig.1.3). Recently, the enzyme cytochrome (cyt) P460 in *Nitrosomonas europaea* AOB was also suggested to convert NH_2OH quantitatively to N_2O under anoxic conditions (Caranto et al., 2016).

Nitrifier denitrification

Nitrifier denitrification is the reduction of NO_2^- to N_2O carried out by AOB (Wrage et al., 2001) (Fig.1.3). NO_2^- was first reduced to NO by copper-containing NIR and then further reduced to N_2O catalyzed by NOR. Due to the lack of genes coding for NOS, N_2O is the end-product of the ND pathway (Shaw et al., 2006). NH_2OH or the cellular pool of reduced electron carriers can act as electron donors for the NO_2^- and NO reduction. ND pathway plays a key role in N_2O production, especially under limited O_2 or elevated NO_2^- conditions. For instance, ND was reported to dominate in N_2O emissions from full-scale nitrifying activated sludge, accounting for 58 to 83% of the total N_2O production (Tallec et al., 2006).

Heterotrophic denitrification

N_2O is an obligate intermediate of HD process. N_2O accumulates due to an imbalance in the activity of nitrogen reducing enzymes under O_2 inhibition, limited organic carbon or NO_2^- accumulation conditions (Wunderlin et al., 2012). This pathway might be as important as the N_2O production by AOB , even in nitrifying systems under very low C/N conditions (Domingo-Félez et al., 2017b).

In addition, heterotrophic denitrifiers possess the genetic potential (i.e. *nosZ* gene) to reduce N_2O to N_2 and with that act as an underlying sink for N_2O. Except for denitrifiers, a variety of organisms also possess the *nosZ* gene, clustered in clade II of *nosZ* phylogeny and often affiliate with Bacteriodetes, Gemmatimonadetes and Deltaproteobacteria (Hallin et al., 2018). Hence, the manipulation of the relative abundance of *nosZ* carrying organisms can increase N_2O consumption rates in microbial communities (Philippot et al., 2011).

Abiotic reaction

The environmentally relevant abiotic reactions include the disproportionation of NH_2OH (Eq. 14), the oxidation of NH_2OH by O_2 (Eq. 15), the oxidation of NH_2OH by HNO_2 (Eq. 16**)**, the reduction of HNO_2 by Fe^{2+} (Eq. 17), the oxidation of NH_2OH oxidation by Fe^{3+} (Eq. 18**)** (Heil et al., 2016; Schreiber et al., 2012; Zhu-Barker et al., 2015). The magnitude of these abiotic N_2O yielding rates are poorly described and hence their contributions to total N_2O production are highly uncertain (Schreiber et al., 2012).

$$4\ NH_2OH \rightarrow 2\ NH_3 + N_2O + H_2O \qquad \text{(Eq. 14)}$$

$$2\ NH_2OH + O_2 \rightarrow N_2O + 3\ H_2O \qquad \text{(Eq. 15)}$$

$$NH_2OH + HNO_2 \rightarrow N_2O + 2\ H_2O \quad \text{(Eq. 16)}$$

$$HNO_2 + 2Fe^{2+} + 2H^+ \rightarrow 2Fe^{3+} + 0.5\ N_2O + 1.5\ H_2O \quad \text{(Eq. 17)}$$

$$4\ Fe^{3+} + 2\ NH_2OH \rightarrow 4\ Fe^{2+} + N_2O + H_2O + 4\ H^+ \quad \text{(Eq. 18)}$$

1.2.2 Operational parameters affecting N_2O production

The documented N_2O emissions in lab-scale and full-scale BNR systems varied between 1- 17% of the NH_4^+ removed (Desloover et al., 2011; Gao et al., 2016; Kong et al., 2013; Lv et al., 2016; Mampaey et al., 2016). The variation might be due to the different responses of N_2O production and consumption pathways to different operational strategies (e.g. feeding and aeration pattern) and parameters (e.g. DO, NO_2^- and pH) (**Paper I**).

DO

DO concentration is considered as an important factor controlling N_2O emission by AOB and denitrifiers (Kampschreur et al., 2009; Tallec et al., 2006). Higher N_2O emission from AOB under limited O_2 availability has been reported in previous studies and ND was suggested as the responsible pathway for the increased N_2O (Kampschreur et al., 2009; Pijuan et al., 2014; Tallec et al., 2006). In contrast, N_2O produced via NN pathway is promoted under higher DO concentrations (Peng et al., 2015a; Wunderlin et al., 2012). For HD pathway, both synthesis and activity of denitrifying enzymes are significantly suppressed by O_2 with NOS as the most oxygen-sensitive. Hence, more net N_2O is accumulated in terms of less consumption during denitrification when oxygen is present in low amounts (Kampschreur et al., 2009; Lu and Chandran, 2010).

NO_2^-

NO_2^- is commonly known to stimulate N_2O production through ND and HD pathways (Cua and Stein, 2011; Law et al., 2012; Todt and Dörsch, 2016). Increased N_2O production by ND in response to high NO_2^- was suspected to reflect the detoxifying mechanisms of AOB (Yu and Chandran, 2010). Beaumont et al. (2004) found that *nirK* gene expression was induced by increasing concentrations of NO_2^- in *N. europaea* AOB. Yu and Chandran (2010) also observed rapid increases of *nirK* and *norB* mRNA concentrations in the presence of high NO_2^- concentrations (280 mg N/L) in *N. europaea* batch cultures. The stimulation effect of NO_2^- on N_2O production in AOB has also been linked to DO dependence (Peng et al., 2015a; Todt and Dörsch,

2016). The stimulating degree of high NO_2^- concentrations on N_2O production was shown to be more important at low DO than at high DO levels (> 1.5mg /L) (Peng et al., 2015a). However, contradictory results were reported in other studies, where exceedingly high NO_2^- did not cause a reduction in N_2O production (Hynes and Knowles, 1984; Law et al., 2013). The possible explanation is an inhibition of the expression of the *nirK* gene above a NO_2^- threshold (Law et al., 2013). In addition, FNA instead of NO_2^- was suggested to be the true substrate in the ND pathway (Shiskowski and Mavinic, 2006). The contradictory conclusions on the effect of NO_2^- on N_2O production remains to be further studied. High NO_2^- concentrations also have been shown to affect the activity of denitrifying enzymes, especially NOS, leading to the accumulation of N_2O (Schulthess et al., 1995; Zeng et al., 2003). The study by Zhou et al. (2008) further demonstrated that FNA rather than NO_2^-, showing 50% and complete inhibition of NOS activity at 0007-0.001 and > 0.004 mg HNO_2- N/L, respectively.

pH

pH is a key controller to achieve nitritation and has a significant impact on the N_2O production of the AOB enriched culture. First, pH affects hydrolysis equilibriums of $NH_4^+ \leftrightarrow NH_3$ (Eq. 19) and $NO_2^- \leftrightarrow HNO_2$ (Eq. 20). An increasing pH shifts NH_4^+ to NH_3, the true substrate for AOB, and decreases FNA concentrations (Anthonisen et al., 1976). High concentrations of FA and FNA are inhibitory to activities of AOB and NOB (Anthonisen et al., 1976; Vadivelu et al., 2007, 2006). Second, pH affects conversion rates of enzymes involved in N-network, as the enzymes have different pH optima (Illanes et al., 2008) (**Paper II**). Hence, pH may cause imbalances between enzymatic reaction steps that lead to the accumulation of intermediates, such as NH_2OH, NO_2^- or NO that could be further biologically or chemically converted to N_2O (**Paper II and III**).

$$NH_4^+ \leftrightarrow NH_3 + H^+ \text{ (pka =9.25 at 25℃)} \quad \text{(Eq. 19)}$$

$$NO_2^- + H_2O \leftrightarrow HNO_2 + OH^- \text{ (pka =3.35 at 25℃)} \quad \text{(Eq. 20)}$$

However, the reported effect of pH on N_2O production is variable under different experimental conditions (Law et al., 2011; Lv et al., 2016; Rathnayake et al., 2015). In the pH range of 6.0-8.5, Law et al. (2011) obtained the maximum N_2O production rate (N_2OR) and ammonium removal rate (ARR) at pH 8, and found that increasing ammonia oxidation activity may promote N_2O production, which was independent of FA and FNA concentrations. Further-

more, Rathnayake et al. (2015) observed the highest N_2O emission at pH 7.5 in PN granules (pH 6.5-8.5), and found no dependence of N_2OR on ARR. In contrast, N_2O emission was found to decrease when the initial pH increased from 7.5 to 8.5 in oxygen-limited PN reactors (Lv et al., 2016). In addition, the kinetics and mechanisms of abiotic reactions are supposed to be highly pH dependent, which adds another dimension of complexity (Bennett et al., 1982; Bothner-By and Friedman, 1952; Hughes and Stedman, 1963; Hussain et al., 1968; Morgan et al., 1968).

pH is also regarded as an important factor affecting net N_2O production during denitrification, which increases with decreasing pH (Pan et al., 2012; Thörn and Sörensson, 1996). Thörn and Sörensson (1996) found that over 40% of the added nitrate accumulated as N_2O at pH 6.0, increasing to almost 100% when pH decreased to below 5 in activated sludge in WWTPs.

Carbon sources

The availability of inorganic carbon is another factor influencing the N_2O production by AOB. A recent study by Peng et al. (2015b) revealed a linear relationship between N_2OR and inorganic carbon concentrations in an enriched nitrifying sludge. The authors attributed lower N_2OR to lower AOB respiration rates under inorganic carbon limitation (Peng et al., 2015b). Limiting availability of biodegradable organic carbon has also been reported to stimulate N_2O emissions during denitrification (Chung and Chung, 2000; Hanaki et al., 1992). The possible reason could be the electron competition between denitrifying enzymes under limiting organic carbon availability (Law et al., 2012).

1.2.3 N_2O mitigation strategies

The ongoing research on N_2O pathways and influencing factors mentioned above provides potential guidelines in the request to minimize N_2O emission in BNR systems. The key to N_2O mitigation is to minimize its production and maximize its consumption (Desloover et al., 2012). The possible mitigation strategies are summarized in Table 1.1. However, as these strategies only have been applied at laboratory-scale, the effectiveness remains to be verified in full-scale trials (Law et al., 2012).

Table 1.1 Overview of N_2O mitigation strategies in BNR system

Objective	Approach
Minimize N_2O production	Ensure stable substrate levels by gradual/step feeding regime, sufficient mixing and buffer volume capacity
	Ensure sufficiently high DO in case of NO_2^- accumulation and constant DO (avoid transient anoxic to oxic changes)
	Ensure low NH_4^+/FA and NO_2^-/FNA
	Ensure neutral and constant pH (avoid transient changes)
	Ensure sufficiently high SRT
Maximize N_2O consumption	Ensure sufficiently high COD/N
	Choose proper carbon source in case of external COD dosage (e.g. N_2O emissions ethanol > methanol)
	Ensure efficient aeration in previous stage (no over-aeration) and provide sufficient anoxic HRT
	Bio-augment with N_2O-consuming heterotrophic denitrifiers *Pseudomonas stutzeri*
	Ensure sufficient copper availability for N_2O reductase synthesis

The table is modified based on Desloover et al. (2012). DO: dissolved oxygen; FA: free ammonia; FNA: free nitrous acid; COD: chemical oxygen demand; SRT: sludge retention time; HRT: hydraulic retention time.

1.3 Research objectives and approaches

In this thesis, the mechanism to achieve stable nitritation performance is proposed, N_2O production dynamics and pathways are identified and quantified, the effect of pH on N_2O production rates and pathways is quantified, and abiotic N_2O production rates and its contributions to overall N_2O emissions in nitritation system are determined. An overview of the research approach followed in this thesis is presented in Fig.1.4.

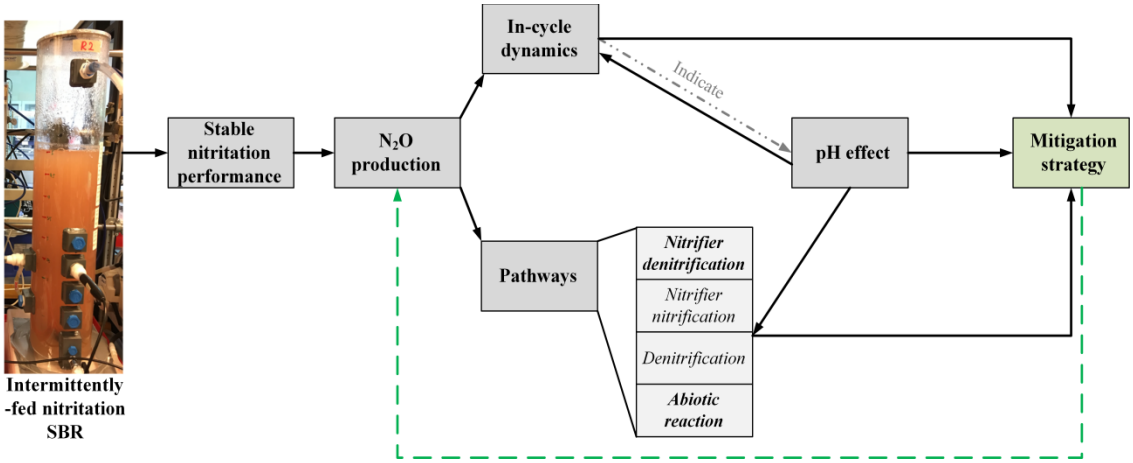

Fig.1.4. Overview of the research approach in this thesis

The specific objectives of this thesis are:

- To identify N_2O dynamics and determine N_2O production rates and pathways in intermittently-fed high-rate nitritation reactors (**Paper I**)
  - Setup and operate two SBRs performing stable nitritation
  - Monitor reactor performance, characterize N_2O dynamics and microbial community composition and determine net N_2O production rates
  - Quantify N_2O production pathways via ^{15}N labeling technique
- To investigate the effect of pH on N_2O production to reduce N_2O production via pH control (**Paper II&III**)
  - Examine current knowledge on pH effect on microorganisms, pathways and enzymes involved in N_2O production
  - Determine N (i.e. NH_4^+, NH_2OH, NO_2^-, NO, N_2O and NO_3^-) conversion rates at different pH campaigns
  - Apply NDHA model to predict N_2O production pathways at different pH values
- To examine the role of abiotic N_2O production in nitritation system (**Paper IV**)
  - Determine N_2O production rates and infer reaction kinetics of all the relevant abiotic reactions
  - Quantify the effect of pH on abiotic N_2O production rates and reaction kinetics
  - Evaluate the contribution of abiotic reactions to overall N_2O emissions from nitritation reactors

1.4 Overview of methods

Throughout the PhD project, two nitritation reactors were operated as duplicates and similar material and methods were applied to meet the objectives of this thesis. The methods mainly consisted of reactor operation pattern, quantification of net N_2O production, additions of stable isotope labeling substrate, pH experiment and abiotic bath tests. For further details, see **Paper I-IV**.

1.4.1 Reactor operation pattern

Two lab-scale SBRs (R1 and R2) with the working volume of 5L were used during the PhD project (Fig.1.5). A 6-h working cycle consisted of 320 min reaction phase including five consecutive intervals of 1 minute feeding followed by a 63 minutes inter-feed period, 30 min settling phase, 5 min decanting phase and 5 min idle phase. The volumetric exchange ratio (VER) was 50%, resulting in a HRT of 12 h. The reactors were operated at room temperature (20-26 °C). Synthetic wastewater containing ammonium bicarbonate (NH_4HCO_3), sodium bicarbonate ($NaHCO_3$) and trace chemicals were used to provide nitrogen, inorganic carbon source and trace elements for the growth of microorganisms. In this work, two reactors were operated aiming to achieve high and stable nitritation performance and low N_2O production.

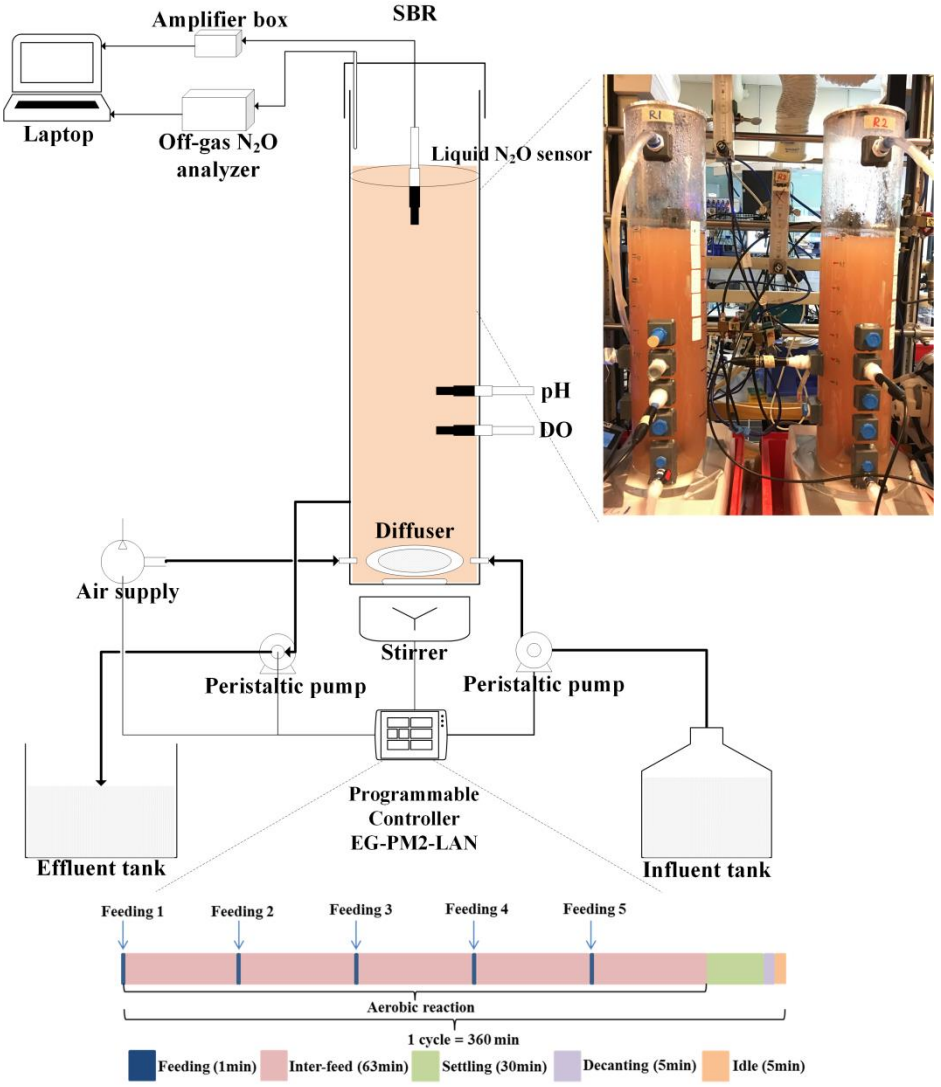

Fig.1.5. Schematic diagram of the setup and operation of SBRs

1.4.2 Quantification of net N_2O production

Liquid phase N_2O was analyzed by a N_2O-R Clark-type microsensor and data was logged every 30s. A gas filter correlation N_2O analyzer that logged data on a minute basis was applied to monitor off-gas N_2O (**Paper I, III**). The liquid phase N_2O concentrations were used for the quantification of N_2O production rates.

The liquid N_2O measured in the reactor is as result of the net rate at which it is produced and its net mass transfer rate (Domingo-Félez et al., 2014). Stripping is also considered in order to interpret liquid profiles at different aeration rates. Hence net N_2O production and emission rates were calculated as follows:

Instantaneous net N_2O production rate , $r_{N_2O_i} = \frac{\Delta N_2O_i}{\Delta t} + k_L a_{N_2O_i} \cdot N_2O_i$ (Eq. 21)

Daily averaged net N_2O production rate, $R_{N_2O} = \sum (r_{N_2O_i} \cdot \Delta t) \times 4 \frac{\text{cycle}}{\text{day}}$ (Eq. 22)

$k_L a_{N_2O,(Q.air=constant)} = A \cdot V_{reactor}^{B} + C$ (Eq. 23)

Where $r_{N_2O_i}$ is the instantaneous net N_2O production rate at time i, $\frac{\Delta N_2O_i}{\Delta t}$ is the differential term of liquid concentration at time i, and $k_L a_{N_2O_i} \cdot N_2O_i$ is the stripping rate at time i, which equals the emission rate. The N_2O volumetric mass transfer coefficient ($k_L a_{N_2O}$) was determined experimentally at different volumes and flow rates scenarios by fitting to a power function (Eq. 23) (**Paper I**). The net N_2O produced per NH_4^+removed ($\Delta N_2O/\Delta NH_4^+$, %) and the specific net N_2O production rate (N_2OR, mg N/g VSS/d) were calculated from the daily averaged net N_2O production rate (Eq. 22).

1.4.3 ^{15}N-labeled substrate additions

The ^{15}N-labeled nitrogen compounds ($^{15}NH_4^+$ and $^{15}NO_2^-$) were separately added at the second feed into nitritation reactors to identify and quantify the microbial sources of N_2O accumulation. The isotopic composition and concentration of N_2O and N_2 were determined by a gas chromatograph-isotope ratio mass spectrometer (Thermo Electron, Delta V advantage system) (Dalsgaard et al., 2012). $^{15}NH_4^+$ and $^{15}NO_2^-$ were analyzed after being converted to N_2 with hypobromite (Warembourg, 1993) and sulfamic acid (Füssel et al., 2012), respectively. $^{15}NO_3^-$ was analyzed, after removal of any

$^{15}NO_2^-$ with sulfamic acid, by cadmium reduction followed by conversion of the NO_2^- product to N_2 with sulfamic acid (McIlvin and Altabet, 2005).

Rates of ^{15}N-labeled N_2O and N_2 production were calculated from the measured excess concentrations of $^{14}N^{15}NO$, $^{15}N^{15}NO$, $^{14}N^{15}N$ and $^{15}N^{15}N$ and the k_La for N_2O and N_2, respectively, similar to the calculations for bulk net N_2O production rate described above.

The total conversion of NH_4^+ and NO_2^- to the gaseous products, irrespective of the pathway, was determined by division of the rate of ^{15}N-labeled gas production (^{15}N-N_2O = $^{14}N^{15}NO$ + 2 x $^{15}N^{15}NO$; ^{15}N-N_2 = $^{14}N^{15}N$ + 2 x $^{15}N^{15}N$) by the labeling fraction F of the substrate (F_A = [$^{15}NH_4^+$] x [NH_4^+]$^{-1}$ and F_N = [$^{15}NO_2^-$] x [NO_2^-]$^{-1}$), e.g.:

$$\text{Rate}(NH_4^+ \rightarrow N_2O) = \text{Rate}(^{15}NH_4^+ \rightarrow {}^{15}N\text{-}N_2O) \quad \text{(Eq. 24)}$$

Production of N_2O through denitrification in the $^{15}NO_2^-$ experiments was calculated in two ways (Eq. 25, 26), both based on the principle of random nitrogen isotope pairing (Nielsen, 1992) and resting on the assumption that denitrification is the only source of double-labeled products with $^{15}NO_2^-$. Here, Eq. 25 represents a rate based on NO_2^- in the bulk liquid only, with a known F_N, and Eq. 26 represents a situation where F_N at the site of reaction may differ from that in the bulk liquid and is instead estimated from the ratio of $^{15}N^{15}NO$ production to $^{14}N^{15}NO$ production, R_{46}:

$$\text{Denitrification}_{N_2O,\ \text{bulk}} = \text{Rate}(^{15}N\ ^{15}NO) \times F_A^{-2} \quad \text{(Eq. 25)}$$

$$\text{Denitrification}_{N_2O,\ \text{coupled}} = \text{Rate}(^{15}N\ ^{15}NO) \times (2R_{46} \times [1+2R_{46}]^{-1})^{-2} \quad \text{(Eq. 26)}$$

1.4.4 pH experiment

The pH experiment was conducted over 80 days (Fig.1.6). pH was controlled at five different values (pH = 6.5, 7, 7.5, 8 and 8.5) in R2. An online pH controller (HACH, Loveland, USA) was used to control pH automatically by dosing 0.5 M $NaHCO_3$ and/or 0.5 M HCl. The reactor was maintained at each pH value for 3-9 days. Before and after each pH change, the reactor was operated without pH control (varying between 7.4 and 7.9) as a control for at least 4 days (named baseline) (Fig.1.6). Before baseline operation, biomass from reactor R1 and R2 was mixed and distributed equally into the two reactors again to allow the microorganisms to recover after potential pH shocks,

to avoid cumulative impacts on microbial activities from previous pH changes, and to maintain similar mixed liquor volatile suspended solid (MLVSS) concentrations during the experimental period.

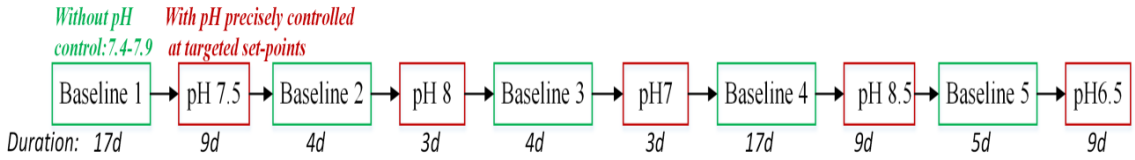

Fig.1.6. Overview of the pH experiment. The experiment period: 25th September, 2017 – 12nd December, 2017.

1.4.5 Abiotic bath tests

The kinetics and stoichiometry of abiotic reactions were quantified in a series of batch tests under different experimental conditions, including pH, absence/presence of oxygen, and reactant concentrations. The tests were conducted in a 0.4 L jacketed glass vessel at room temperature (24-26℃) in deionized water (diH_2O) or synthetic medium under oxic (8-8.4 mg/L) or anoxic (0-1 mg/L) conditions. The composition of synthetic medium was the same as used for the reactor influent. Before each test, the diH_2O or synthetic medium was saturated with N_2 or air, and adjusted to target pH. The vessel was then completely filled with saturated diH_2O or synthetic medium and sealed with the insertion of rubber stoppers and sensors (N_2O, DO and pH). Different amount of substrates were then added into the vessel to initiate abiotic reactions after sensor stabilizations. During batch tests, pH was controlled by manually dosing 0.5 M HCl or 0.5 M $NaHCO_3$, and continuous mixing was provided with a magnetic stirrer at 100 rpm. Two types of experimental scenarios were used:

Scenario 1: Parallel tests were conducted at fixed initial pH (pH 4, 5, 6, 7, 8 and 9) and fixed initial substrates concentrations (17.8 mM NO_2^-, 0.07 mM NH_2OH, 0.5 mM Fe^{2+} and 0.5 mM Fe^{3+}).

Scenario 2: Tests were performed at certain initial concentrations with stepwise changes (increase in reactants and decrease in pH) by sequentially spiking reactants and acid.

2 Achievement of stable high-rate nitritation reactor

Even though it remains a challenge to maintain stable nitritation over the long-term and minimize N_2O emissions from nitritation systems. Two intermittently-fed SBRs were operated with high-rate nitritation performance and low N_2O production for 710 days in this PhD project. The mechanisms to achieve high & stable nitritation performance and low N_2O production are discussed in this section.

2.1 Reactor performance

Nitritation performance

The reactors displayed stable NH_4^+ removal at the end of phase 1 and phase 2. With stepwise increases in loading from 0.3 to 0.8 g N/L/d during phase 2, the specific ARR of 0.46 ± 0.09 and 0.5 ± 0.02 g N/g VSS/d were obtained in R1 and R2, respectively, while the average ammonium removal efficiency (ARR/ammonium loading rate (ALR)) remained nearly stable at 86 ± 11% (R1) and 88 ± 8% (R2) (Fig.2.1 and Table 2.1). High nitrite accumulation efficiency (nitrite accumulation rate (NiAR)/ARR) of 92 ± 17% (R1) and 93 ± 14% (R2) were maintained at the end of phase 1 and throughout phase 2. NO_3^- accumulated at low concentrations throughout the whole operation period (Fig.2.1). The observations of high NO_2^- accumulation and insignificant NO_3^- production indicated that NOB were successfully outcompeted by AOB in the reactors. Furthermore, an average AOB/NOB ratio of > 200 at the end of phase 1 and during phase 2 further confirmed efficient suppression of NOB and enrichment of AOB (**Paper I**).

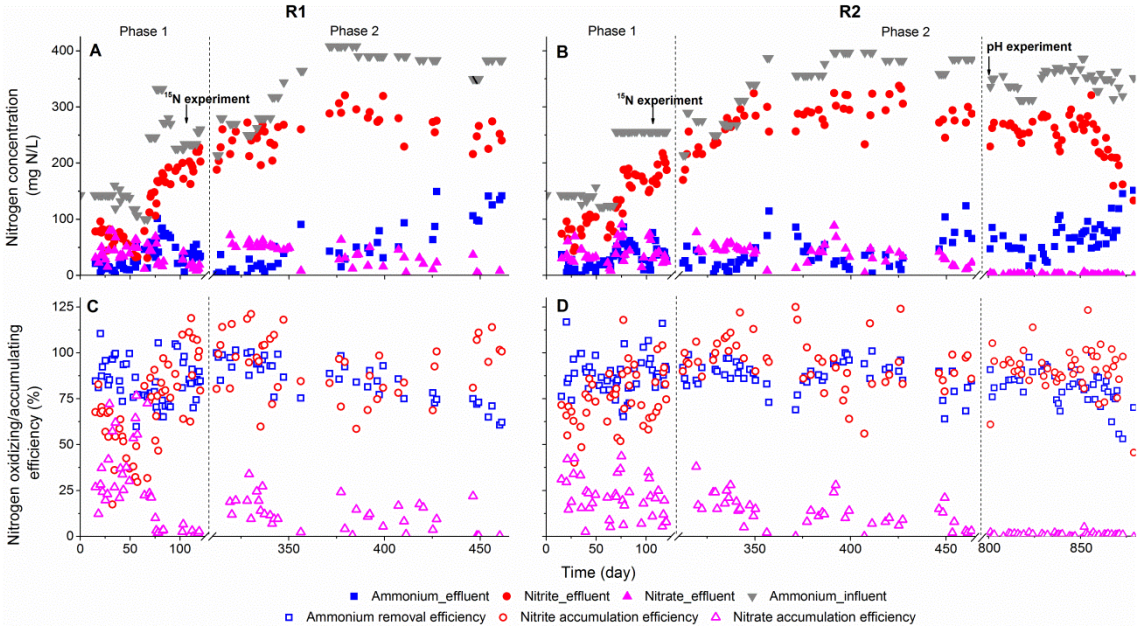

Fig.2.1 Nitritation performance in R1 (A, C) and R2 (B, D) throughout the operational period. (A, B) Nitrogen concentrations (NH_4^+, NO_2^-, NO_3^- in effluent, NH_4^+ in influent). (C, D) Nitrogen conversion efficiency (ammonium removal efficiency (ARR/ALR), nitrite accumulation efficiency (NiAR/ARR), nitrate accumulation efficiency (NaAR/ARR)). The first break at the x-axis represents a period of 170 days, when the reactors were stopped and biomass was stored at 4°C. The second break at the x-axis represents a period of 338 days prior to the pH experiment, when the reactors were continuously operated.

N_2O production

Throughout the whole experiment period, the average specific net N_2OR varied in the range of 5.9-8.4 and 10.2-16 mg N/g VSS/d in R1 and R2, respectively (Table 2.1). The differences in the specific N_2OR between the two reactors could be due to different MLVSS concentrations of the biomass in the reactors. The net N_2O production in both reactors corresponded well with the genetic potential for N_2O production as with the ratio of *nirS* plus *nirK* over *nosZ*-targeted genes far above 1 (**Paper I**). The average net N_2O produced per NH_4^+ removed ($\Delta N_2O/\Delta NH_4^+$) during phase 2 was almost 3 times higher compared to the end of phase 1 in both reactors. We speculate that new microbes with higher expression of the nitrifier-denitrification pathway may be selected during the long-term operation under elevated NO_2^-or the cultured microbes adapted to higher NO_2^-, resulting in higher expression of the pathway, and with that higher N_2O production. Yet, this assumption requires further phylogenetic analysis of the microbial community. Furthermore, the contribution of each feed period to the total N_2O production of a cycle was not

equal, as N_2O gas escaping after feed 1 (23 to 41%) was considerable higher than the emissions of the other feeds (Table 2.1).

Table 2.1 Overview of ARR, N_2OR and $\Delta N_2O/\Delta NH_4^+$ in R1 and R2 during phase 1 and 2. The net N_2O produced during each feed is stated as the percentage of total net N_2O production during the entire cycle. pH experiment was conducted on day 801–879.

Reactor	R1		R2		
	Phase 1	Phase 2	Phase 1	Phase 2	
	Day 106–112	Day 395–451	Day 106–112	Day 397–463	Day 801–879
ARR (g N/L/d)	0.5 ± 0.05	0.60 ± 0.05	0.5 ± 0.02	0.76 ± 0.06	0.35-0.67
ARR (g N/g VSS/d)	1.04 ± 0.11	0.46 ± 0.09	1.78 ± 0.08	0.50 ± 0.02	0.93-1.25
$N_2OR \times 10^3$ (g N/g VSS/d)	5.9 ± 1.8	8.4 ± 3.5	16.0 ± 5.9	10.2 ± 3.5	12.0-84.7
$\Delta N_2O/\Delta NH_4^+$ (%)	0.6 ± 0.2	2.0 ± 1.0	0.8 ± 0.3	2.1 ± 0.7	1.1-7
Feed 1 (%)	23 ± 5	41 ± 9	30 ± 5	27 ± 5	27 ± 6
Feed 2 (%)	22 ± 1	14 ± 2	21 ± 2	17 ± 2	19 ± 7
Feed 3 (%)	19 ± 1	15 ± 2	18 ± 2	18 ± 2	19 ± 7
Feed 4 (%)	17 ± 2	16 ± 2	16 ± 2	19 ± 1	18 ± 7
Feed 5 (%)	18 ± 3	15 ± 4	15 ± 2	21 ± 5	17 ± 7
# cycles	n=22	n=23	n=22	n=20	n=135

The N_2O production factors of ca. 2% are in the low range of previous reports for both lab-scale and full-scale PN systems, ranging between 1–17% (Desloover et al., 2011; Gao et al., 2016; Kong et al., 2013; Lv et al., 2016; Mampaey et al., 2016). This is the first study to obtain low N_2O emissions at such high nitritation efficiencies. Low DO and high NO_2^- conditions are commonly known to stimulate N_2O production (Peng et al., 2015a, 2014). However, N_2O emissions measured under DO below 0.5 mg/L and NO_2^- over 250 mg N/L were much lower than other lab-scale PN SBRs operated under low DO and high NO_2^- conditions (N_2O emission factor up to 17%) (Gao et al., 2016; Lv et al., 2016). With the intermittent feeding strategy at low DO, NH_3 oxidation could be maintained at relatively low rates, which has previously been shown to reduce N_2O emissions from BNR systems (Domingo-Félez et al., 2014; Law et al., 2011). For instance, Law and coworkers (2011) achieved a substantial reduction in N_2O production by decreasing feeding rate from 1 L/2.5 min to 1 L/25 min during the reaction phase without affecting the nitritation performance. Instead of reducing the feeding rate, we operated nitritation reactors with five intermittent feedings within a cycle. This step-feed strategy has previously been suggested as an effective optimization approach to reduce N_2O emissions from SBRs (Mavrovas, 2014; Yang et al.,

2009, 2013). Hence, intermittent feeding was postulated as the cause for the low N_2O emission from high-performance nitritation system here.

2.2 Mechanisms to achieve high and stable nitritation performance

Various parameters (e.g. DO, FA, FNA, temperature and feeding strategy) have been reported to affect the selective enrichment of AOB over NOB (Blackburne et al., 2008; Hellinga et al., 1998; Liu and Wang, 2014; Vadivelu et al., 2007; Yang et al., 2013). Due to low DO level (< 0.5 mg/L) in two nitritation SBRs, oxygen limitation was an important factor for achieving high NiAR/ARR over 93% in both reactors. Regarding FA inhibition, a five-fold increase of FA concentrations to 3.1 ± 0.8 mg NH_3-N/L at the end of phase 1 could be the reason for a sharp drop in nitrate accumulation (Fig.2.1). However, FA did not fully inhibit the activity of NOB at any time in our study and also likely did not affect AOB activities within the observed FA concentration. Compared to the reported inhibitory concentration (0.02-0.2 mg HNO_2-N/L), FNA of 0.008 ± 0.002 mg HNO_2-N/L in reactors was too low to have a negative effect on NOB activities. Furthermore, no evidence of NO_2^- inhibition on AOB was obtained despite of high NO_2^- accumulation up to 323 mg N/L. ARR was observed to positively correlate with NO_2^- concentrations, which agrees with a previous study with mixed microbial communities showing high NH_4^+ oxidation to NO_2^- (150−160 mg NO_2^--N/h/g VSS) at NO_2^- concentrations up to 1000 mg N/L (Law et al., 2013). FNA concentrations in reactors remain much below reported inhibitory concentrations of 0.4 mg HNO_2-N/L for AOB (Vadivelu et al., 2007, 2006). Although moderate temperatures (20-26°C) applied during reactor operation was much lower than optimal temperatures (30-35°C) required for selective removal of NOB over AOB (Hellinga et al., 1998; Yang et al., 2007), we still achieved the efficient competitiveness of AOB over NOB, resulting in high nitritation efficiency from day 78 onwards. In addition, long-term high-rate nitritation has not been reported yet in intermittently fed SBRs, while high nitrite accumulation efficiency of 85% and > 95% for 150 and 174 days, respectively, was previously reported in step-feed A/O SBRs (Lemaire et al., 2008; Yang et al., 2007).

However, it is often difficult to maintain stable nitritation over the long-term period even in successfully established nitritation systems (Bernet et al.,

2001; Fux et al., 2004; Villaverde et al., 2000; Yang et al., 2013). For example, the nitrite accumulation efficiency of submerged nitrifying biofilters decreased from 65% to 30% after 6 months as NOB became adapt to high FA (Villaverde et al., 2000); a transient increase of DO in a two-stage PNA reactor was observed to induce a transition from stable nitritation for more than 100 days to complete nitrification within 2 days (Bernet et al., 2001). Nevertheless, we successfully operated two SBRs with high nitritation performance and high AOB abundance for 710 days. The operational strategies applied, i.e. intermittent feeding together with low DO set-points, were suspected to enable long-term high-rate nitritation in the two reactors.

3 Identification and quantification of N_2O production dynamics and pathways

During the operation period, N species (NH_4^+, NO_2^-, NO_3^-, NH_2OH, NO and N_2O) were monitored to identify in-cycle dynamics and determine N conversion rates, while *in situ* applications of ^{15}N labeled NH_4^+ or NO_2^- were used to quantify N_2O production pathways. In this section, in-cycle N dynamics and flux are presented and the dominant pathway in nitritation reactors is revealed.

3.1 In-cycle N dynamics and flux

The patterns of in-cycle dynamics of N species over the reaction phase were very reproducible during the whole period for both reactors (Fig.3.1).

pH and DO

pH varied in the range of 7.4-7.9 under normal operational conditions as well as baseline operation during the pH experiment. After each feed, pH transiently increased due to the bicarbonate and phosphate content of the influent, while pH decreased during the inter-feed periods due to proton release during nitritation (**Paper I&III**). During the controlled pH campaigns, pH was maintained at the targeted set-point (**Paper III**). DO concentrations remained almost stable during the reaction phase except for a transient and slight increase (ca. 17%) after each feeding due to the oxygen dissolved in the influent.

NH_4^+/FA and NO_2^-/FNA

NH_4^+ concentration increased by approximately 50% after each feeding while NO_2^- concentration decreased due to dilution. FA and FNA concentrations followed the changes of NH_4^+ and NO_2^- at different pH values.

NH_2OH

NH_2OH was always detected during the pH experiment: its concentration increased rapidly after feeding and remained almost constant during inter-feed periods. The measured NH_2OH concentrations of 0.05 ± 0.01 mg N/L were consistent with values documented in lab-scale PN reactors (0.03-0.11 mg N/L) (Kinh et al., 2017; Soler-Jofra et al., 2016). Bulk liquid NH_2OH concentrations are the result of the balance between production associated to ARR

and consumption from conversion of NH_2OH to NO_2^- or to N_2O either biologically or chemically. Even low NH_2OH concentrations are still compatible with significantly high N_2OR since the turnover of NH_2OH may be high (Soler-Jofra et al., 2016). As a potential toxic intermediate and the primary energy yielding substrate during NH_3 oxidation, NH_2OH typically did not accumulate except transiently immediately after feedings, when short-term elevated ARR were measured (Fig.3.1-D). This finding is consistent with the studies by Yu et al. (2017) and Liu et al. (2017). In these studies, the authors observed an immediate accumulation of NH_2OH after the activation of NH_3 oxidation during the anoxic-to-oxic transition. In addition, a 0.3-0.7% of instantaneous NH_2OH:NO_2^- ratio detected after feeding in our experiment is comparable with the value (0.1-0.6 %) reported for AOB pure cultures during early phases of the incubation experiments (Liu et al., 2017).

NO

The observation of fairly constant NO concentrations during aerated reaction phase and the increase in NO concentration after the aeration stopped was consistent with previous studies on AOB pure cultures or in PNA systems (Kampschreur et al., 2008; Yu et al., 2010; Yu and Chandran, 2010). Under anoxic conditions, NO is suspected to be generated via ND, NN and HD pathways (Caranto et al., 2016; Yu et al., 2017; Yu and Chandran, 2010). NO could be produced via ND process by using NO_2^- as an electron acceptor indicated by over-expressed NIR and under-expressed AMO, HAO and NOR in *N. europaea* cultures during anoxia (Yu et al., 2010). HAO or cyt P460 mediated NH_2OH oxidation was also identified as an importance source of NO after imposing anoxic conditions (Caranto et al., 2016; Yu et al., 2017). This is confirmed by approximately 20% drop of NH_2OH concentrations during settling phases in our study. Besides, due to the limited organic carbon availability in the reactor, heterotrophic denitrifiers might accumulate NO and N_2O during anoxic phase (Chung and Chung, 2000). A possible explanation for substantial reduction of NO concentrations upon to aerated reaction phase is that the presence of O_2 would inhibit both ND and HD pathway and also decrease NO production via NN pathway as NO can rapidly react with O_2 to form NO_2^- instead of N_2O. The observation of an unsystematic correlation between NO and N_2O as well as other N compounds implies that transcription of enzymatically sequential pathways in the AOB metabolism was independent with a high degree of flexibility and versatility in overall energy transduction (Yu et al., 2017, 2010).

N_2O

Liquid and off-gas N_2O profiles over the reaction phase were very reproducible during the whole operational period for both reactors. In-cycle N_2O profiles had the following pattern: an initial maximum in N_2O concentration occurred when the first feed initiated, after which the concentration declined until the next feeding; another four smaller peaks in N_2O concentration were observed in the subsequent feedings. The maximum net N_2O production after the first feed was mainly due to N_2O accumulation during the non-aerated settling phase (Fig.3.1). Heterotrophic denitrifiers might be responsible for this N_2O accumulation, which is then released at the onset of aeration (Itokawa et al., 2001). The genetic potential for N_2O production by denitrifiers was present through the high relative abundance of *nirS* (**Paper I**). Similar observations have also been described in other studies, where 60-70% of the quantified N_2O emission was attributed to the anoxic N_2O formation in PN reactors (Mampaey et al., 2016; Rodriguez-Caballero and Pijuan, 2013). Furthermore, the transiently increase in net N_2O production rates with the rise in pH after each feeding pulse indicated a potential effect of pH on N_2O production during the reaction phase (Fig.3.1). Compared to insignificant changes in DO, a transient increase (ca. 80%) in FA caused by rising NH_4^+ and pH (primary reason) after each feeding was observed. High FA availability might lead to high metabolic rates during periods of high N flux, as reflected by transient peaks of ARR, hydroxylamine accumulation rate (NhAR) and NiAR (Fig.3.1-D). Consequently, more substrates (e.g. NH_2OH and NO_2^-) and electrons (produced by NH_2OH oxidization) were available to potentially promote N_2O productions. Thus, pH appears a potential important variable affecting N_2O dynamics and emission factors, which is discussed in details in chapter 4.

N conversion rates

Based on bulk concentrations, in-cycle conversion rates of N species were calculated (Fig.3.1-D). ARR, NiAR and NhAR peaked transiently after each feeding (2-4 times higher, $p < 0.05$) and were almost constant during the inter-feed period. There was always a positive net production of N_2O and NO over a cycle: N_2OR increased after each feeding and decreased during the inter-feed period ($p < 0.05$), whilst NOR remained unchanged ($p > 0.05$) and close to zero.

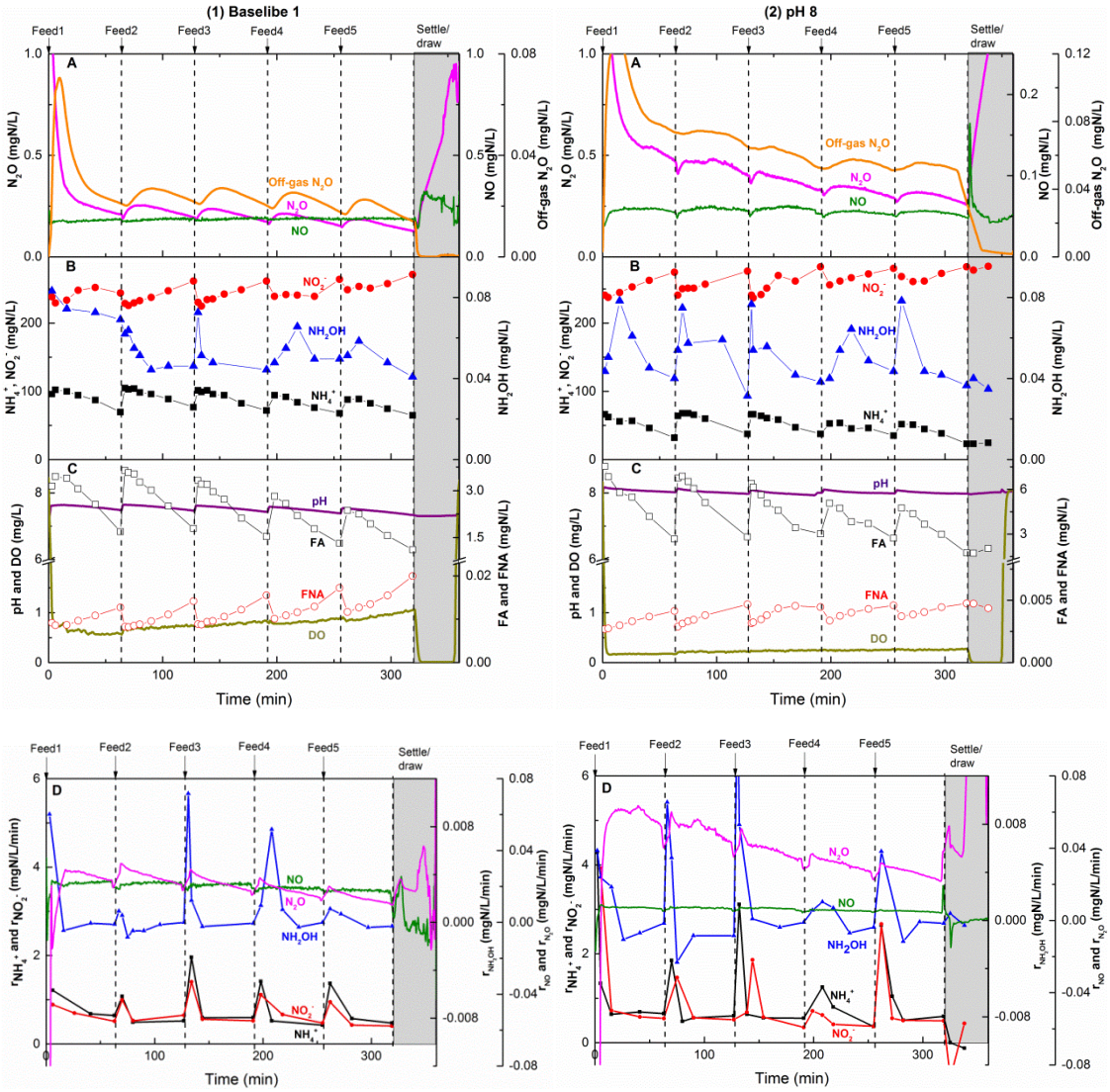

Fig.3.1 In-cycle dynamics and conversion rates of N species at Baseline 1 (1) and 8 (2) during the pH experiment. (A) Liquid and off-gas N_2O and liquid NO concentrations. (B) Bulk concentrations of NH_4^+, NO_2^- and NH_2OH. (C) pH, DO, calculated FA and FNA. (D) Conversion rates of N species (NH_4^+, NO_2^-, NH_2OH, NO and N_2O). The calculation of conversion rates is based on in-cycle concentrations of N species; each point represents the slope of 2-3 concentration points within a certain time period. Abbreviations: free ammonia (FA), free nitrous acid (FNA).

N mass balance

The mass balance during feed and inter-feed period at different pH values and baselines was calculated by normalizing the amount of produced NH_2OH, NO_2^-, N_2O and NO (mg N) to the amound of NH_4^+ removed (mg N) (Fig.3.2). There was approximately 70% of the removed NH_4^+ converted to NO_2^- during feed period while the ratio increased to ca. 93% during inter-feed period.

NH_2OH production accounted for 0.16-0.63% of the removed NH_4^+ after feedings but it was barely observed during inter-feed period. In spite of the observed peaks of N_2O and N_2OR after feedings, a larger fraction of the NH_4^+ removed was converted to N_2O during inter-feed period. A possible explanation could be that ARR increased or decreased (3 times) faster than N_2OR in feed or inter-feed period.

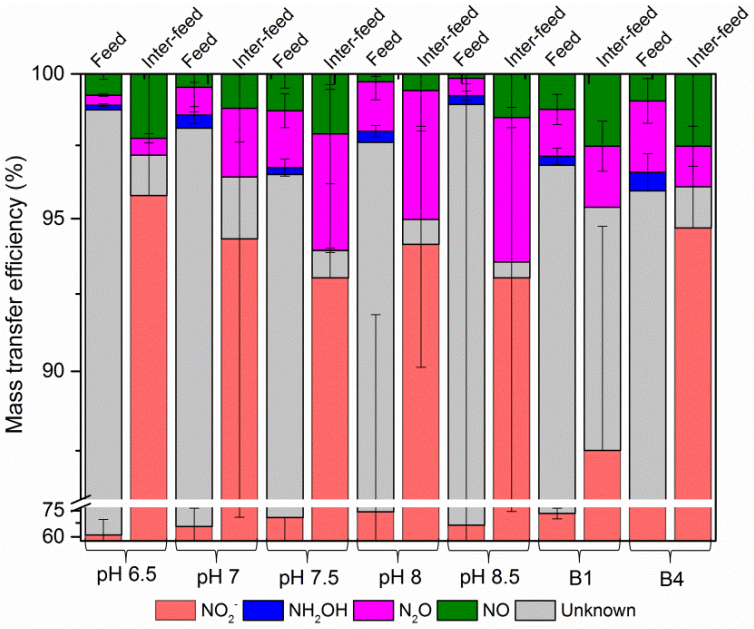

Fig.3.2 N mass balance during feed and inter-feed period at different pH values and baselines during the pH experiment. No significant NO_3^- concentrations were detected (<10 mg N/L), thereby the data was not shown in the figure. The calculation of average and standard deviation was based on data during feed 2-5 (n=4).

3.2 Nitrifier denitrification as the dominant pathway

After the addition of ^{15}N-labeled substrates, the label was transferred to both N_2O and N_2 within 2-3 minutes, regardless of whether ^{15}N was added as $^{15}NO_2^-$ or $^{15}NH_4^+$ (Fig.3.3). The dynamics of ^{15}N-N_2O mirrored those of bulk N_2O, and N_2O was the dominant product in $^{15}NO_2^-$ incubations accounting for 57–58% of the labeled N_2O + N_2 in both feedings, while it was only 17–23% with $^{15}NH_4^+$. The production of ^{15}N-N_2O from $^{15}NO_2^-$ corresponded to a total conversion of NO_2^- to N_2O of 5.7–9.9 µg N/g VSS/min, which was more than 3 times higher rate of N_2O production from $^{15}NH_4^+$ (**Paper I**). The results implied ND as the dominant source of N_2O in two nitritation reactors, which was consistent with general understanding that this pathway is favored by

low DO and high NO_2^- conditions (Colliver and Stephenson, 2000; Kampschreur et al., 2008; Peng et al., 2015; Tallec et al., 2006).

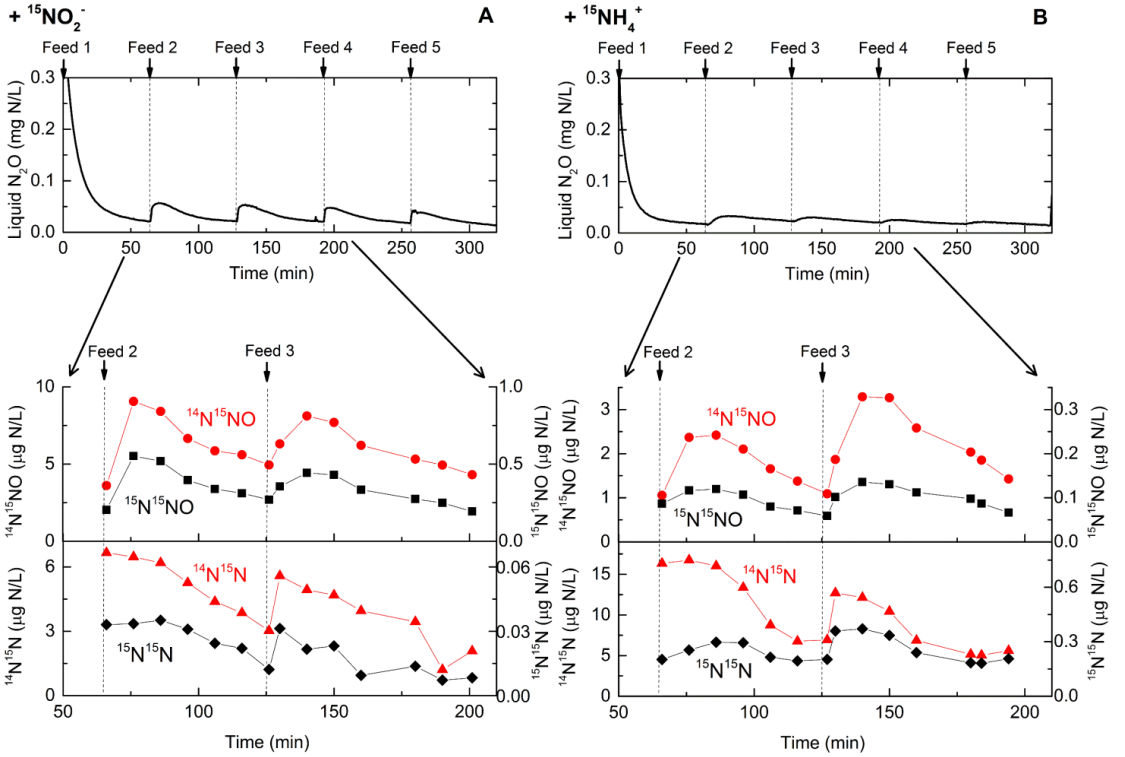

Fig.3.3. Bulk liquid N_2O concentrations during the reaction phase of one cycle (upper panels) and isotopically labeled N_2O and N_2 concentrations during feed 2 and 3 (lower panels) in Reactor 1. $^{15}NO_2^-$ spikes were performed at 111 days (A) and $^{15}NH_4^+$ spiked at 107 days (B).

The ^{15}N-labeling technique cannot distinguish ND from HD. However, several pieces of evidence pointed to the ND pathway: (1) the increasing N_2O production by each NH_4^+ feeding indicated NH_4^+ dependence rather than heterotrophy; (2) the observed ratio and pattern of N_2O and N_2 production rates did not match the typical characteristic of heterotrophic denitrification, like N_2O production rates were much higher than N_2 production rates from NO_2^-, whereas N_2O is generally a minor byproduct of heterotrophic denitrification (Betlach and Tiedje, 1981); (3) the very low ratio of $^{15}N^{15}N$ to $^{14}N^{15}N$, differing markedly from the $^{15}N^{15}NO$:$^{14}N^{15}NO$ ratio in N_2O, indicated that another process involves in N_2 production from NO_2^-. As AOB have not been reported to produce N_2, this suggests the involvement of anammox bacteria, which were indeed detected in the biomass in low abundance (**Paper I &III**). Compared to the theoretical 1:1 pairing of N from NH_4^+ and NO_2^- in anammox (van de Graaf et al., 1995), we obtained ca. 2.5 fold higher production from

$^{15}NH_4^+$ than from $^{15}NO_2^-$ during ^{15}N experiments. Potential explanations for the imbalance in rates could be either a close coupling of nitritation and anammox that requires a physical association of AOB and, or a variation in anammox rates between the two series of experiments that were conducted 5 days apart. In addition, isotope pairing calculations showed that NO_2^- during its reduction to N_2O was mixed most likely with unlabeled nitrogen from NH_4^+. N_2O was hypothesized to be produced via ND process with part of the newly-formed NO_2^- shunted directly to reduction either intracellullarly or within cellular aggregates before mixing completely with NO_2^- in the bulk liquid. Alternatively, the combination of N from NH_4^+ and NO_2^- could occur at the level of NO if this compound is a free intermediate during ammonium oxidation (Stein, 2011).

4 The effect of pH on N_2O production rates and pathways

pH can have a significant effect on the N_2O production in nitritation systems by influencing dissociation equilibriums (e.g. $NH_4^+ \leftrightarrow NH_3$ and $NO_2^- \leftrightarrow HNO_2$) or imbalancing enzymatic reaction steps leading to an accumulation of intermediates (e.g. NH_2OH, NO_2^- or NO) that could be further biologically or chemically converted to N_2O. However, the knowledge on pH effect is incomplete, partly due to involvement of different enzymes and multiple pathways, and partly due to the direct and indirect effects of pH on various central processes (signaling or transcriptional and post-transcriptional phenomena) in bacterial cells (Blum et al., 2018; Law et al., 2011). The application of mathematical modeling will contribute to unraveling the effect of pH on N_2O production pathways and the reduction of N_2O emissions through pH set-point management. Hence, a wide range of pH conditions (pH 6.5-8.5) were imposed on the nitritation reactor to examine the effect of pH on overall N_2O production rates and comprehensive NDHA model was applied to quantify the effect of pH on N_2O production pathways.

4.1 N conversion rates at varying pH set-points

The effect of pH on overall N_2O production rates was quantified in the nitritation reactor from pH 6.5 to 8.5 at pH interval of 0.5. While pH was precisely maintained at the targeted set-point during controlled pH campaigns (Fig.4.1-A), it varied between 7.4 and 7.9 during baseline operation without pH control. DO concentrations were almost constant within a cycle and similar at different pH levels (0.6 ± 0.1 mg O_2/L), except at pH 6.5 (2.2 ± 0.5 mg O_2/L) and pH 8 (0.2 ± 0.03 mg O_2/L) (Fig.4.1-A). MLVSS concentrations were 0.5 ± 0.1 g/L and particle size distribution (PSD) was similar during the experimental period with average particle size of 205 ± 29 µm (Fig.4.1-B). Bulk NH_4^+ and NO_2^- concentrations were 74 ± 39 mg N/L and 233 ± 39 mg N/L, respectively, resulting in FA and FNA concentrations in the range of 0.5-15 and 0.001-0.065 mg N/L, respectively. NH_2OH concentrations were around 0.05 ± 0.01 mg N/L while NO_3^- concentrations were below 10 mg N/L (Fig.4.1-D, E). Under similar NH_4^+ loading rates (0.7 ± 0.03 g N/L/d), ammonium removal efficiency (ARR/ALR) decreased from 93% at pH 8 to 53% at pH 6.5. In contrast, the reactor displayed stable nitrite accumulation effi-

ciency (NiAR/ARR) above 90% across the tested pH range (Fig.4.1-C). Using baseline operation, reactor performance was allowed to recover with an average ARR/ALR and NiAR/ARR of 84 ± 6% and 92 ± 2%, respectively (**Paper III**).

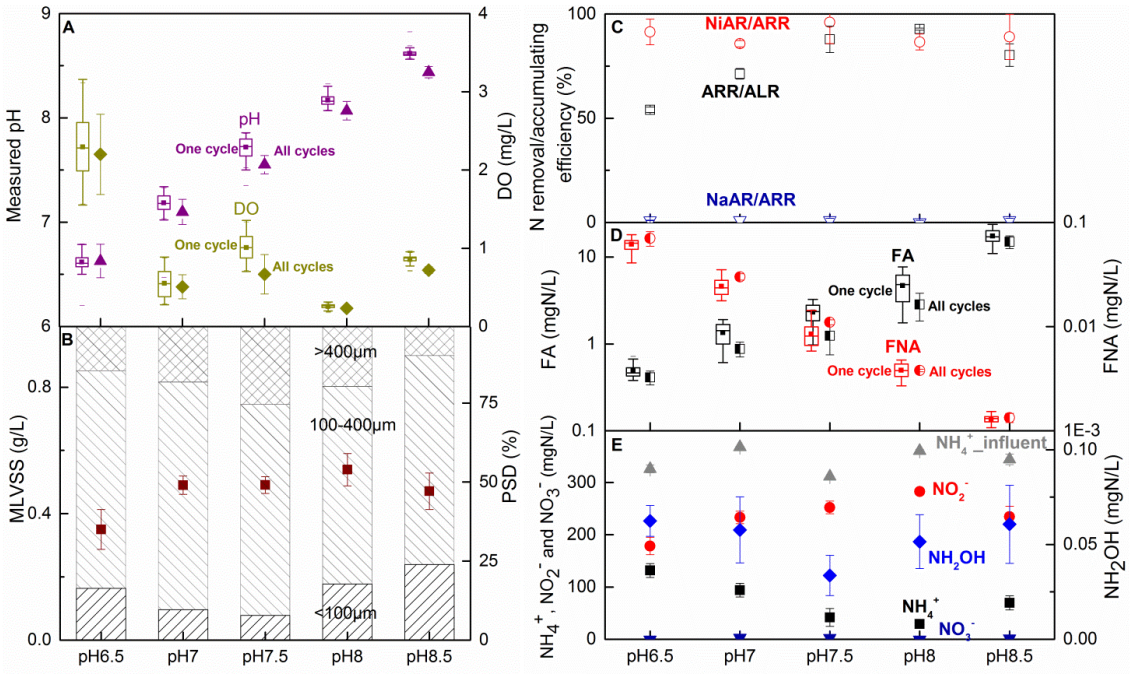

Fig.4.1. Overview of reactor performance during the pH experiment. (A) Measured pH and DO averaged over one cycle and all cycles. (B) MLVSS and PSD of biomass. (C) Nitrogen conversion efficiency (ARR/ALR, NiAR/ARR and NaAR/ARR). (D) Calculated FA and FNA within one cycle and all cycles. (E) Bulk concentrations of NH_4^+, NO_2^-, NO_3^- and NH_2OH. Each data point represents the average of all measurements at the same pH level (n>6). Error bars indicate standard deviations of measurements.

The specific ARR remained almost constant at 1.2 ± 0.2 g N/g VSS/d across the examined pH range ($p > 0.05$) (Fig.4.2-A). No significant changes of NiAR and NOR with pH were observed ($p > 0.05$), while NhAR decreased as pH increased from 6.5-8 and then increased slightly at pH 8.5 ($p < 0.05$). The specific N_2OR and $\Delta N_2O/\Delta NH_4^+$ increased with pH from 6.5 to 8, and decreased slightly at pH 8.5 ($p < 0.05$) (Fig.4.2). The maximum specific N_2OR and $\Delta N_2O/\Delta NH_4^+$ at pH 8 were 0.08 ± 0.01 g N/g VSS/d and 7.0 ± 1.3%, respectively. However, the specific N_2OR dropped by almost half at the end of baseline operation, which was associated with higher DO concentrations in the reactor.

The observed effects of pH on N_2OR in our experiment were in agreement with previous reports: Rathnayake et al. (2015) and Kinh et al. (2017) ob-

served unchanged ARR between pH 6.5 and 8.5 in PN reactors but highest N_2O emission at pH 7.5 and 7, respectively; Law and coworkers (2011) reported highest N_2OR and ARR at pH 8 (pH were examined from 6.0 to 8.5) and a positive linear correlation relationship between N_2OR and ARR. On the contrary, Lv et al. (2016) found that both ARR and N_2OR decreased with increasing initial pH from 7.5 to 8.5 in a PN reactor. Specifically, HD was regarded as dominant pathway and its contribution to total N_2O emissions decreased from 69 % at pH 7.5 to 40% at pH 8.5 (Lv et al., 2016). Different observations on pH effects are probably caused by different predominant N_2O production pathway, i.e. ND in our study and HD in Lv et al. (2016), which responded differently to pH changes. Compared to the short-term batch tests or transient pH changes in previous studies (where responses were measured minutes or hours afterwards), here we imposed longer-term reactor-scale pH campaigns (i.e. the reactor was operated at each pH value for 3-9 days). Hence, in this study microorganisms may have acclimated to new pH changes and reach stable nitritation activities.

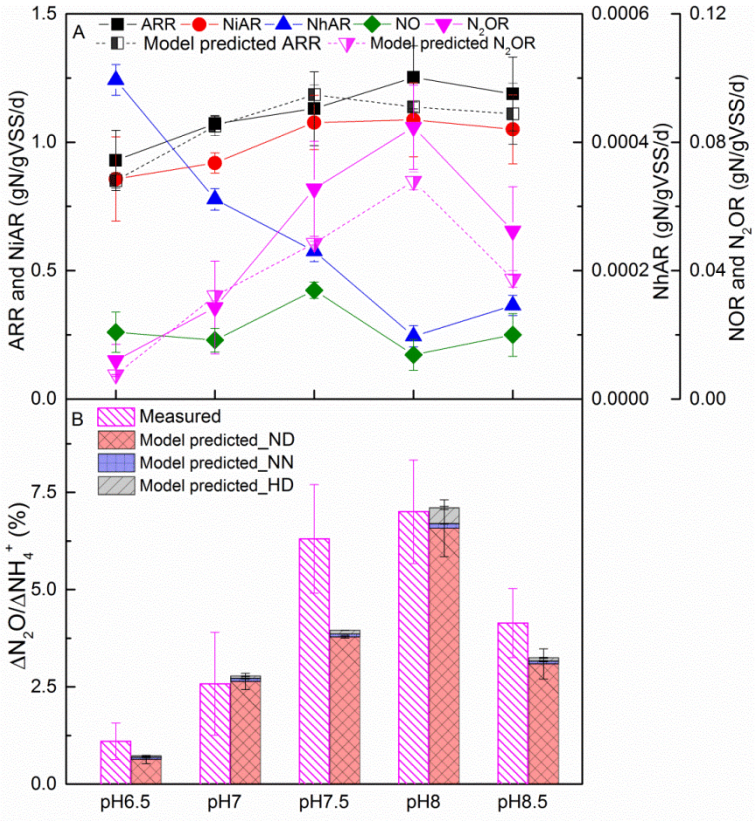

Fig.4.2. The effect of pH on the specific N conversion rates (A) and $\Delta N_2O/\Delta NH_4^+$ (B) (n=5-29). Abbreviations: ammonium removal rate (ARR), nitrite accumulation rate (NiAR), hydroxylamine accumulation rate (NhAR), net NO production rate (NOR),

net N_2O production rate (N_2OR), nitrifier denitrification (ND), nitrifier nitrification (NN) and heterotrophic denitrification (HD).

4.2 Model-based estimation of N_2O production pathways at varying pH set-points

A previously proposed consilient model (i.e. NDHA) that comprehensively described ND, NN and HD pathways was applied to interpret experimental observations. The NDHA model was calibrated via off-line extant respirometric assays using biomass sampled from the reactors (Domingo-Félez et al., 2017a). The default parameters were first validated and further optimized to adequately describe O_2 consumption and ARR, then N_2OR and $\Delta N_2O/\Delta NH_4^+$ at different pH set-points. Based on global sensitivity analysis, the most sensitive parameters (in order of sensitivity) were $\mu_{AOB.AMO}$, $K_{AOB.NH3}$ and $K_{AOB.I.NH3}$ for ARR, and η_{NIR}, $K_{AOB.I.O2.}$, $K_{AOB.NH2OH.ND}$, $K_{AOB.HNO2}$ and $K_{AOB.I.HNO2.ND}$ for N_2OR. These parameters were estimated by fitting the experimental data of DO, ARR, N_2OR and $\Delta N_2O/\Delta NH_4^+$ at different pHs and during baseline operation. The estimated values are comparable with values reported in literature (Hiatt and Grady, 2008; Ni et al., 2014; Park et al., 2010). The average errors between experimentally measured and model predicted ARR and DO at different pH levels and two baselines were below 10% (R^2 = 0.82 and 0.91, respectively, F-test = 1), indicating a good model fit. The model was also able to capture the effect of pH on N_2OR and $\Delta N_2O/\Delta NH_4^+$ across pH levels (R^2 = 0.85 and 0.80, respectively, F-test = 1). The model predicted an increase in N_2OR and $\Delta N_2O/\Delta NH_4^+$ via ND pathway as pH increased from 6.5 to 8 and then decreased at pH 8.5 (Fig.4.2-B). N_2OR via NN pathway was predicted to follow a similar (but less significant) trend as ND pathway. Predicted N_2O production rates and consumption rates via HD pathway were insignificant compared to overall N_2OR (< 3%). At all tested pH levels, N_2OR from ND pathway dominated other pathways, contributing 87-96% of total N_2O production in the reactor (Fig.4.2-B). The best-fit simulated relative contributions of different pathways to N_2O production did not change significantly across the tested pH range.

Here, we present a comprehensive study on the effect of pH (6.5-8.5) on N_2O production in the nitritation reactor. Higher pH was found to significantly stimulate specific N_2O production rates mainly via ND pathway. Hence, operating nitritation systems at slightly acidic or neutral pH (which still permit sufficient microbial activity) can reduce N_2O production by up to seven-fold.

5 Abiotic N_2O production rates and the contribution to overall N_2O emissions in nitritation reactors

During the nitritation process, the reactive intermediates, such as NH_2OH and NO_2^-, may engage in chemical reactions resulting in N_2O production (Schreiber et al., 2012). However, these abiotic reactions were ignored or deemed unimportant in most previous studies on N_2O production in BNR reactors, due to low environmental concentrations, high reactivity or short lifetimes of reactive nitrogen intermediates (Zhu-Barker et al., 2015). The magnitude of these different abiotic N_2O yielding rates are poorly described, and hence their contributions to total N_2O production are highly uncertain (Schreiber et al., 2012). Here, we quantify the kinetics and stoichiometry of the relevant abiotic reactions in a series of batch tests under different and relevant conditions, including pH, absence/presence of oxygen, and reactant concentrations.

5.1 Abiotic N_2O production rates and reaction kinetics

NH_2OH disproportionation and/or oxidation by O_2

Alkaline over acidic pH and synthetic medium instead of diH_2O enhanced N_2O formation by NH_2OH disproportionation plus oxidation, whilst oxygen showed a limited stimulatory effect on N_2O production (Fig.5.1-A). At initial NH_2OH concentration of 0.07 mM, the maximum NH_2OH depletion rate (r_{NH2OH}) (0.0073 mM/h) was obtained at pH 8 in synthetic medium under oxic conditions (Fig.5.1-B). Only 21 ± 7% of the removed NH_2OH was recovered in produced N_2O, indicating side reactions and yields did not vary substantially with pH (Fig.5.1-C).

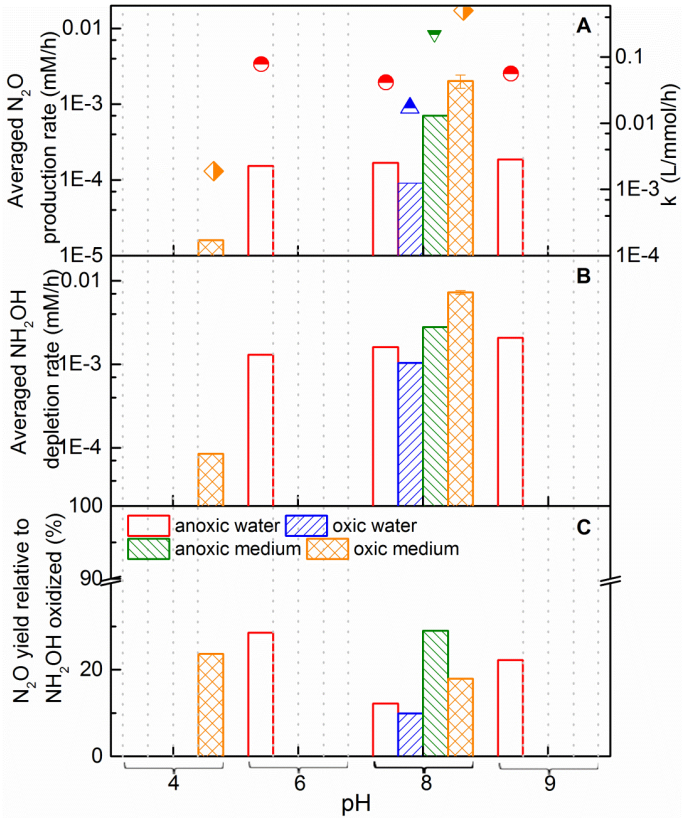

Fig.5.1. NH_2OH disproportionation and/or oxidation by O_2 at different pH values (Scenario 1). (A) Averaged N_2O production rate (bar) and rate constant (k) (scatter); (B) Averaged NH_2OH depletion rate; (C) N_2O yield relative to NH_2OH oxidized (%). Gray dot bars represent the tests that were not performed. Error bars indicate standard deviations of measurements.

The reaction of NH_2OH with HNO_2

The N_2O production was initiated immediately after addition of NH_2OH and NO_2^- into the vessel and terminated due to the complete depletion of NH_2OH (**Paper IV**). At excess NO_2^- concentrations (≥17.8 mM), HNO_2 concentrations remained nearly constant, ranging from 0.00018-0.9 mM depending on pH (4.5-8) and were unlikely to limit the reaction. After addition of NO_2^-, NH_2OH was depleted at a rate of 0.00026-0.39 mM/h and N_2O was produced at a rate of 0.00019-0.78 mM/h at different pH set-points, DO levels and medium types (Fig.5.2-A, B). The r_{NH2OH} and r_{N2O} showed a strong dependency on pH. The N_2O production rate increased 4 order of magnitude, with a consistent (almost 4 log) decrease in pH (Fig.5.2-A). Furthermore, in the sequential acid addition scenario, sequential pH drops resulted in a rapid N_2O production, with r_{N2O} at pH 6 being more than two orders of magnitude higher than at pH 8.5. The results suggested HNO_2 instead of NO_2^- as the actual re-

actant. According to the measured NH_2OH, HNO_2 and N_2O and assumed reaction kinetics (Eq. 16), rate constant (k) was calculated in the range of 3.3-56 L/mmol/h, with higher value at lower pH ($k=8272.5e^{-1.1pH}$, $R^2 = 0.99$) (Fig.5.2-A). Similar to r_{N2O}, r_{NH2OH} significantly increased with decreasing pH, which was ca. 400 times higher at pH 4.5 than at pH 8 (Fig.5.2-B). Oxygen availability and medium type showed limited effects on either NH_2OH depletion or N_2O formation (Fig.5.2-B). The influence of the reactant (NH_2OH/HNO_2) concentration on the reaction kinetics was minimal, and outweighed by the pH effect. The increase of N_2O yield relative to NH_2OH oxidize from 35% at pH 8 to nearly 200% at pH 4.5 clearly indicated different reaction mechanisms involved under different pH levels (Fig.5.2-C).

Early investigations have suggested that the reaction of NH_2OH with HNO_2 occurs by an initial O-nitrosation, which presumably leads to the formation of $ON{\cdot}NH_2{\cdot}OH^+$ (Hughes and Stedman, 1963). Then $ON{\cdot}NH_2{\cdot}OH^+$ will readily tautomerise to a mixture of cis- and trans-hyponitrous acids, where largely are cis-hyponitrous acids that decompose rapidly to N_2O and water, leaving a small amount of the stable trans-form (Bothner-By and Friedman, 1952; Hughes and Stedman, 1963; Hussain et al., 1968). The equations of $r_{N2O} = k{\cdot}[NO_2^-]{\cdot}[NH_2OH]$ or $k{\cdot}[HNO_2]{\cdot}[NH_2OH]$ or $k{\cdot}[H^+]{\cdot}[HNO_2]{\cdot}[NH_2OH]$ have been reported to describe the reaction (Bennett et al., 1982; Bothner-By and Friedman, 1952; Döring and Gehlen, 1961; Harper et al., 2015; Hughes and Stedman, 1963). This reaction is assumed to be first order in HNO_2 (Bennett et al., 1982), though the order in HNO_2 was found to increase above 1 or even approach 2 in extreme cases at low acidities (pH=2) (Hughes and Stedman, 1963). Moreover, the rate constant has been shown to depend on acidity (Bennett et al., 1982; Hughes and Stedman, 1963). For example, Bennett et al. (1982) found that k value increased with $H^+ < 2$ M (pH -0.3) but decreased with H^+ at 2 M of H^+. The dependence of k value on acidity might be due to a change in rate-determining step from the nitrosation step to the transfer of the NO^+ group from oxygen to nitrogen (Bennett et al., 1982), or the effect of pH on the decomposition or rearrangement of $ON{\cdot}NH_2{\cdot}OH^+$ (Hughes and Stedman, 1963; Hussain et al., 1968). Since the pH range (4-9) tested in our experiments was far above pH -0.3, the observation of decreasing k values at more alkaline pH agrees with the observations by Bennett et al. (1982).

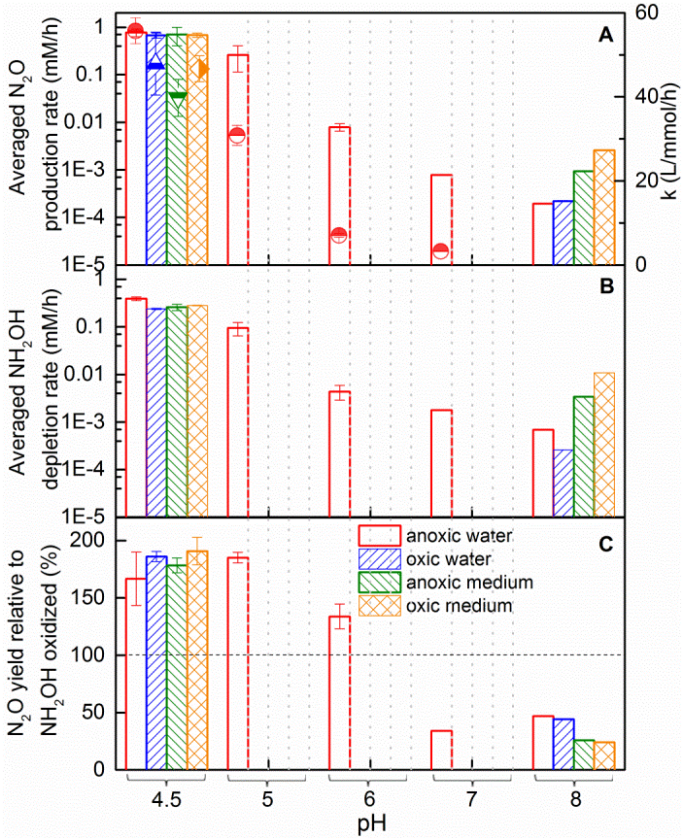

Fig.5.2. Reaction of NH_2OH with HNO_2 at different pH values (Scenario.1). (A) Averaged N_2O production rate (bar) and rate constant (k) (scatter); (B) Averaged NH_2OH depletion rate; (C) N_2O yield relative to NH_2OH oxidized (%). Gray dot bars represent the tests that weren't performed. Error bars indicate standard deviations of measurements.

The reduction of HNO_2 by Fe^{2+}

After the addition of NO_2^-, Fe^{2+} was linearly oxidized to Fe^{3+} coupled with N_2O formation. Fe^{2+} was depleted at a rate of 0.28 ± 0.04 mM/h while Fe^{3+} was accumulated at a rate of 0.29 ± 0.02 mM/h at pH 4.5, which was consistent with equimolecular conversion (Eq. 17). Furthermore, twofold higher Fe^{2+} depletion rate (r_{Fe2+}) than r_{N2O} and close to 100% of N_2O yield to Fe^{2+} oxidized indicated that Fe^{2+} reacted with HNO_2 following the stoichiometry of Eq. 17. The observation of steep increases of Fe^{2+} depletion and N_2O emission after HCl spikes suggested that both r_{Fe2+} and r_{N2O} were strongly dependent on pH, whilst there were no significant responses to increasing concentrations of NO_2^- and Fe^{2+}.

The oxidation of NH_2OH by Fe^{3+}

The reaction of NH_2OH with Fe^{3+} was only tested at pH 4.3 because of the formation of precipitates and iron oxyhydroxide species at alkaline pH, which would have resulted in lower rates. Fe^{3+} and NH_2OH were depleted at rates of 0.005 and 0.003mM/h, respectively, resulting in production rates of Fe^{2+} and N_2O of 0.005 and 0.001mM/h, respectively. The result verified that Fe^{3+} reacted with NH_2OH the stoichiometry of Eq. 18 as expected.

5.2 pH as the key factor influencing abiotic N_2O production

pH shows a significant effect on abiotic N_2O reaction kinetics in the presence of HNO_2, NH_2OH and iron (Fe^{2+} and Fe^{3+}) (**Paper IV**). First, pH affects the speciation of NO_2^- ($NO_2^- + H_2O \leftrightarrow HNO_2 + OH^-$), NH_2OH ($NH_2OH + H_2O \leftrightarrow NH_3OH^+ + OH^-$) and iron (via the formation of various iron oxyhydroxide species of varying solubilities). However, previous studies on abiotic N_2O reactions in nitritation systems were not able to conclude whether NO_2^- or HNO_2 was the actual reactive species (Harper et al., 2015; Kampschreur et al., 2011; Terada et al., 2017). In our experiments, sharp N_2O peaks were observed after pH drops that shifted NO_2^- to HNO_2, whilst sequential spiking of NO_2^- did not significantly stimulate N_2O formation, indicating HNO_2 instead of NO_2^- as the actual reactant. Combined with the observed dependence of the reaction rate constant on pH ($k=8272.5e^{-1.1pH}$, $R^2 = 0.99$), we suspected that more acidic pH would enhance the N_2O production through affecting both rate constants and NO_2^- speciation. With respect to NH_2OH disproportionation and oxidation by O_2, r_{NH2OH} and r_{N2O} was lower at more acidic pH as NH_2OH can be ionized as NH_3OH^+ and is thermally stable under acidic conditions (Ma et al., 2017).

pH also affects conversion ratios of final products of abiotic reactions (Fig.5.1, 5.2). The conversion of the oxidized NH_2OH into N_2O was 35± 9% at pH ≥ 7 and 174 ± 19% of at pH < 7, indicating that more complicated or side reactions might exist under different pH values. The low recovery of N_2O at pH ≥ 7 was consistent with findings by Soler-Jofra et al. (2018, 2016), where the conversion ratio varied from 20 ± 1% to 40 ± 2% at pH 7.5. The authors attributed this to the presence of a side reaction between NH_2OH and HNO (one intermediate of reaction (1)) with N_2 as the final product. The higher theoretical recovery of N_2O at acidic pH has not been previously re-

ported. Since NH_2OH was completely oxidized at the end of tests, the gap in the N mass balances cannot be explained by equimolecular NH_2OH and HNO_2, and the excessively recovered N_2O was suspected to be contributed by a higher stoichiometry in HNO_2. However, N_2O could not be detected in the sole presence of HNO_2 (data not shown). The transient N_2O peaks were observed immediately after acid additions (Scenario 2), making r_{N2O} difficult to estimate. Considering low sensitivities of the N_2O sensor towards changes in pH, oxygen and stir intensity, the observation of transient N_2O peaks is unlikely caused by uneven mixing or transient response of N_2O sensor or signal interfered by pH. The determination of abiotic N_2O production rates during sequential acid additions has not been reported yet, which requires further investigation.

5.3 The contribution of abiotic N_2O production in nitritation system

Based on the estimated reaction rate constants (Fig.5.1, 5.2) and the measured NH_2OH and HNO_2 concentrations in the nitration reactor during operation, rates of abiotic N_2O production through the oxidation of NH_2OH by HNO_2 and NH_2OH disproportionation plus oxidation by O_2 were estimated. Then, the relative contribution of abiotic reactions to total N_2O production was estimated at different pH conditions (Table 5.1). The abiotic contributions accounted for less than 3% of total N_2O produced, and showed dependency on pH, increasing from 0.03% at pH 8 to 2.6% at pH 6.5. On the contrary, studies by Soler-Jofra et al. (2018, 2016), Harper et al. (2015) and Terada et al. (2017) concluded that both abiotic and biotic routes contribute in a comparable degree to N_2O emissions (at pH 7) (Table 5.1). For example, using the same operational conditions as observed in a nitritation (i.e., without biomass but at consistent NH_2OH and NO_2^- concentrations of 0.02 mM and 0.0029 mM, respectively), abiotic reaction of NH_2OH with NO_2^- was estimated to contribute 34% of the total N_2O emission in a SHARON reactor (Soler-Jofra et al., 2016). From the estimated k values in this study and experimental conditions in literature, abiotic r_{N2O} were estimated at 1-2 orders of magnitude lower than that originally documented (Table 5.1). The much higher abiotic r_{N2O} measured could be caused by the incorrect quantifying method: Soler-Jofra et al. (2016) used the maximum instantaneous rate to represent overall N_2O production while the initial rate (estimated by linear approximation of initial N_2O concentration profile) was applied in Terada et al. (2017). Hence,

we contend that the significance of abiotic N_2O production has been severely overestimated in recent studies.

To the best of our knowledge, this is the first study that comprehensively quantifies N_2O production by all abiotic chemical reactions at the relevant conditions to nitritiation bioreactors. Abiotic reactions, including NH_2OH oxidation by HNO_2, Fe^{3+}, and O_2 and HNO_2 reduction by Fe^{2+}, could contribute to N_2O production with the reaction of NH_2OH and HNO_2 as the major source. pH was identified as the most significant factor affecting N_2O production rates and the rate constant. N_2O production from the reaction of NH_2OH with HNO_2 was stimulated at acidic pH and HNO_2 instead of NO_2^- was the reactant. Abiotic N_2O production was estimated to contribute < 3% of total N_2O produced in typical nitritation reactor systems (pH 6.5-8) and would only become important at extremely acidic pH ($\leq$ 5). Hence, nitritation reactors were recommended to operate at circum-neutral pH to avoid N_2O accumulation via abiotic reactions at extreme acidic pH and also N_2O produced via biological pathways at more alkaline pH. The significance of abiotic N_2O emissions might be overestimated in recent abiotic studies in nitritation systems. Therefore, correct quantification of abiotic reaction kinetics based on rate constants and careful consideration of pH effects are required to assess the role of abiotic N_2O production in BNR systems.

Table 5.1 The contribution of abiotic reactions to overall N_2O production in nitritation reactors.

Reference	Total N_2O production in nitritation reactors						Abiotic N_2O production				
	Experimental condition					Measured total N_2O production rates (mM/h)	Considered abiotic reaction [c]	Method [d]	Estimated abiotic N_2O production rates (mM/h) [e]		Fraction of abiotic pathway to total N_2O production (%)	
	Reactor types	pH	NH_2OH (mM)	HNO_2 (mM)	NO_2^- (mM)				Original Estimation	Estimation based on this study	Original Estimation	Estimation based on this study
This study	Lab-scale, nitritation, SBR	6.5 [a]	0.0045±0.0006 [a]	0.0046±0.0009 [a]	11.7±1.8 [a]	0.006±0.002 [a]	Eq. 16	Abiotic batch tests without biomass	0.0002±0.00004		2.6±1.2	
							Eq. 14,15		0.000002±0.0000004		0.03±0.01	
		7.0 [a]	0.0041±0.0012 [a]	0.0021±0.0002 [a]	16.6±0.9 [a]	0.02±0.01 [a]	Eq. 16		0.00003±0.000009		0.2±0.09	
		8.0 [a]	0.0037±0.0010 [a]	0.0003±0.000008 [a]	20.2±0.1 [a]	0.07±0.013 [a]	Eq. 16		0.000018		0.03±0.005	
							Eq. 14,15		0.0000002±0.00000006		0.0003±0.0001	
Terada et al. (2017)	Bath tests with AOB enriched biomass	7	0.07-1.4 [b]	0.006 [b]	28.6 [b]	0.2-3.3 [b]	Eq. 16	Abiotic batch tests with/without biomass	0.05-0.9 [b]	0.0014-0.028 [b]	23-44 [b]	0.7-0.8 [b]
Soler-Jofra et al. (2016)	Full-scale, PN, SHARON, flocs	7	0.0043 [b]	0.0029 [b]	46.4 [b]	0.017 [b]	Eq. 16	Abiotic batch tests without biomass	0.006 [b]	0.00004 [b]	34 [b]	0.24 [b]
Harper et al. (2015)	Bath tests with AOB enriched biomass	7	0.007-1.4 [b]	0.006 [b]	28.6 [b]	0.015-0.88 [b]	Eq. 16	Abiotic batch tests with/without biomass and combined with model simulations	0.02-0.7 [b]	0.00014-0.028 [b]	/	0.9-3.2 [b]

[a] Numbers were retrieved from the pH experiment.
[b] Numbers were calculated based on original data in literatures.
[c] Eq. 14 and 15 represent NH_2OH disproportionation and/or oxidation by O_2; Eq. 16 represents NH_2OH oxidation by HNO_2
[d] The details of experimental methods refer to materials and methods section and Table S1, S3 in **Paper IV**.
[e] Estimated abiotic N_2O production rates was calculated based on the equation of $r_{N2O} = k \cdot [HNO_2] \cdot [NH_2OH]$.

6 Conclusions

This PhD project investigated dynamics, identified pathways, and explored mitigation options for N_2O production in nitritation reactors. Two lab-scale intermittently-fed SBRs were operated towards high-rate nitritation performance and simultaneously low N_2O emissions. Stable ^{15}N-labeling technique was applied to quantify N_2O production pathways. Experiments across a range of controlled pH (6.5-8.5) conditions in the nitritation reactor were conducted to examine the effect of pH on N_2O production rates. The effect of pH on pathway contribution was analyzed by the NDHA model. Abiotic N_2O production mechanisms and associated reaction kinetics were investigated in a series of batch tests under relevant conditions with special attention to the effect of pH. Finally, operational conditions were optimized to reduce N_2O emissions from nitritation reactors. Central findings are summarized below:

- Two lab-scale SBRs were operated with the intermittent feeding for 710 days and displayed high nitritation performance with over 93% of the oxidized NH_4^+ converted to NO_2^-. The observations of high NO_2^- accumulation and insignificant NO_3^- production indicated that NOB were successfully outcompeted by AOB in the reactors. An average AOB/NOB ratio of > 200 at the end of phase 1 and during phase 2 further confirmed efficient suppression of NOB and enrichment of AOB.
- The patterns of in-cycle dynamics of N species over the reaction phase were very reproducible during the whole period for both reactors. Conversion rates of NH_4^+, NO_2^-, NH_2OH and N_2O increased transiently after pulse feedings and declined until the next feeding, while NO remained unchanged within the cycle.
- The averaged net N_2O production factor of 2% was in the low range of previous reports for PN systems. The low DO combined with intermittent feeding was sufficient to maintain high nitritation rates, while intermittent feeding may be an effective approach to minimize N_2O emissions from nitritation systems.
- *In situ* application of ^{15}N labeled substrates revealed ND pathway as the dominant pathway of N_2O production.
- The specific ARR and NiAR remained nearly constant across the examined pH range (6.5-8.5) ($p > 0.05$). The specific N_2OR and the fractional N_2O yield ($\Delta N_2O/\Delta NH_4^+$) increased with pH from 6.5 to 8 and decreased slightly with further pH increase to pH 8.5 ($p < 0.05$). N_2O production and

consumption by heterotrophic denitrifiers and abiotic N_2O production were insignificant compared to overall N_2O production.

- NDHA model predicted ND pathway dominated other pathways at all examined pH levels, contributing 87-96% of total N_2O production. ND pathway was responsible for increasing N_2O production at more alkaline pH. The relative contributions of different pathways to N_2O production did not change significantly across the tested pH range.
- The highest abiotic N_2O production rates were measured for NH_2OH oxidation by HNO_2, followed by HNO_2 reduction by Fe^{2+}, NH_2OH oxidation by Fe^{3+}, and finally NH_2OH disproportionation plus oxidation by O_2.
- Compared to other examined factors, pH was identified as the most significant factor, affecting abiotic N_2O production rates and the rate constant. N_2O production from the reaction of NH_2OH with HNO_2 was enhanced at acidic pH and HNO_2 instead of NO_2^- is the reactant.
- Abiotic N_2O production was insignificant during nitritation process across pH 6.5-8 (<3% of total N_2O production) but would only become important at extremely acidic pH ($\leq$ 5). We contend that the significance of abiotic N_2O production was overestimated in previous studies
- In consideration of the effects of pH on both abiotic and biotic N_2O production, we recommend operating nitritation reactors at circum-neutral pH to minimize overall N_2O emissions.

7 Future perspectives

In this study, the efficient suppression of NOB and enrichment of AOB with an average AOB/NOB ratio of > 200 was achieved in two nitritation reactors. However, the exact abundances of different microbial species were not analysed. Monitoring predominant AOB is of significance as changes in AOB species with different physiological properties could determine emission levels of N_2O. The higher N_2O production in phase 2 than phase 1 was speculated due to the selection of species with higher expression of ND pathway during the long-term operation under elevated NO_2^-. Furthermore, regardless of similar microbial community indicated by qPCR analysis during the pH experiment, pH fluctuations may shift the dominant species in microbial community leading to changes in N_2O production. Consequently, quantification of predominant AOB will provide a deeper understanding of effects of operational conditions, like NO_2^- accumulation and pH, on N_2O emissions in nitritation systems.

Heterotrophic denitrifiers might be responsible for N_2O accumulation during the non-aerated settling phase and also contribute to N_2O emission under low DO conditions during aerobic reaction phase. However, it is a challenge to elaborate the individual contribution of denitrification solely with bulk N_2O measurements due to the co-occurrence of ND and HD under similar NO_2^- and DO conditions in mixed culture biomass. Further studies (e.g. using $^{15}N/^{18}O$ stable labeling method) are needed to evaluate respective contributions of different pathways and investigate how pH affects each pathway individually. Moreover, heterotrophic bacteria or the *nosZ* gene carrying microorganisms also offer opportunities to mitigate N_2O emissions from nitritation or PNA systems. Further understanding of the regulation of transcription of the denitrification genes and relevant process parameters for enriching the *nosZ* gene will be beneficial to reduce net N_2O production.

FA and FNA concentrations have reported to inhibit the metabolisms of AOB and NOB, yet, the critical values reported in these studies were variable. The inhibitory effects of FA and FNA might vary with different predominant species of AOB and NOB and different operational conditions. The measurement of inhibitory values of FA and FNA on biomass will provide support for the design and operation of nitritation systems and improve the accuracy and precision of model prediction.

N_2O production rates were showed to increase with pH in the range of 6.5-8 and decreased slightly at 8.5, and ND pathway was predicted as the key contributor for stimulated N_2O production at alkaline pH. Further investigations through the use of transcription analysis and ^{15}N stable isotope labelling are required to directly quantify the pH effect on enzyme activities, functional gene expressions and pathways involved in N_2O production. More specifically, a better understanding of the effect of pH on cellular electron pool and different electron carriers or on ND rates in general is also relevant.

Acidic pH was found to strongly enhance abiotic N_2O production rates and reaction rate constant, yet the exact mechanisms behind remain unclear. Additionally, side reactions were indicated to coexist with these abiotic reactions. Hence, further studies on the mechanistic basis for these observations will provide clearer guidelines for modelling predictions and mitigation strategies.

This study has identified operational strategies via intermittent feeding and pH control as means to mitigate N_2O emission from nitritation systems. However, the potential application of feeding pattern and pH control for N_2O mitigation in full-scale WWTPs remains to be verified and combined with economic and environmental assessment.

8 References

Agrawal, S., Seuntjens, D., Cocker, P. De, Lackner, S., Vlaeminck, S.E., 2018. Success of mainstream partial nitritation/anammox demands integration of engineering, microbiome and modeling insights. Curr. Opin. Biotechnol. 50, 214–221. doi:10.1016/J.COPBIO.2018.01.013

Anthonisen, A., Loehr, R., Prakasam, T., Srinath, E., 1976. Inhibition of Nitrification by Ammonia and Nitrous Acid. J. Water Pollut. Control Fed. 48, 835–852.

Arnaldos, M., Kunkel, S.A., Stark, B.C., Pagilla, K.R., 2013. Enhanced heme protein expression by ammonia-oxidizing communities acclimated to low dissolved oxygen conditions. Appl. Microbiol. Biotechnol. 97, 10211–10221. doi:10.1007/s00253-013-4755-7

Beaumont, H.J.E., Lens, S.I., Reijnders, W.N.M., Westerhoff, H. V., Van Spanning, R.J.M., 2004. Expression of nitrite reductase in Nitrosomonas europaea involves NsrR, a novel nitrite-sensitive transcription repressor. Mol. Microbiol. doi:10.1111/j.1365-2958.2004.04248.x

Bennett, M.R., Maya, L., Brown, G.M., Posey, F.A., 1982. Oxidation of hydroxylamine by nitrous and nitric acids. Inorg. Chem. 21, 2461–2468. doi:10.1021/ic00136a066

Bernet, N., Dangcong, P., Delgenès, J.-P., Moletta, R., 2001. Nitrification at Low Oxygen Concentration in Biofilm Reactor. J. Environ. Eng. 127, 266–271. doi:10.1061/(ASCE)0733-9372(2001)127:3(266)

Betlach, M.R., Tiedje, J.M., 1981. Kinetic explanation for accumulation of nitrite, nitric oxide, and nitrous oxide during bacterial denitrification. Appl. Environ. Microbiol. 42, 1074–1084.

Blackburne, R., Yuan, Z., Keller, J., 2008. Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor. Biodegradation 19, 303–312. doi:10.1007/s10532-007-9136-4

Blum, J.-M., Su, Q., Ma, Y., Valverde-Pérez, B., Domingo-Félez, C., Jensen, M.M., Smets, B.F., 2018. The pH dependency of N-converting enzymatic processes, pathways and microbes: effect on net N 2 O production. Environ. Microbiol. 20, 1623–1640. doi:10.1111/1462-2920.14063

Bothner-By, A., Friedman, L., 1952. The Reaction of Nitrous Acid with Hydroxylamine. J. Chem. Phys. 20, 459–462. doi:10.1063/1.1700442

Bougard, D., Bernet, N., Chèneby, D., Delgenès, J.-P., 2006. Nitrification of a high-strength wastewater in an inverse turbulent bed reactor: Effect of temperature on nitrite accumulation. Process Biochem. 41, 106–113. doi:10.1016/J.PROCBIO.2005.03.064

Caranto, J.D., Lancaster, K.M., 2017. Nitric oxide is an obligate bacterial nitrification intermediate produced by hydroxylamine oxidoreductase. Proc. Natl. Acad. Sci. 114, 8217–8222. doi:10.1073/pnas.1704504114

Caranto, J.D., Vilbert, A.C., Lancaster, K.M., 2016. Nitrosomonas europaea cytochrome P460 is a direct link between nitrification and nitrous oxide emission. Proc. Natl. Acad. Sci. 113, 14704–14709. doi:10.1073/pnas.1611051113

Chung, Y.-C., Chung, M.-S., 2000. BNP test to evaluate the influence of C/N ratio on N_2O

production in biological denitrification. Water Sci. Technol. 42, 23–27. doi:10.2166/wst.2000.0354

Colliver, B.B., Stephenson, T., 2000. Production of nitrogen oxide and dinitrogen oxide by autotrophic nitrifiers. Biotechnol. Adv. 18, 219–232. doi:10.1016/S0734-9750(00)00035-5

Cua, L.S., Stein, L.Y., 2011. Effects of nitrite on ammonia-oxidizing activity and gene regulation in three ammonia-oxidizing bacteria. FEMS Microbiol. Lett. 319, 169–175. doi:10.1111/j.1574-6968.2011.02277.x

Daims, H., Nielsen, J.L., Nielsen, P.H., Schleifer, K.H., Wagner, M., 2001. In situ characterization of Nitrospira-like nitrite-oxidizing bacteria active in wastewater treatment plants. Appl. Environ. Microbiol. 67, 5273–84. doi:10.1128/AEM.67.11.5273-5284.2001

Dalsgaard, T., Thamdrup, B., Farías, L., Revsbech, N.P., 2012. Anammox and denitrification in the oxygen minimum zone of the eastern South Pacific. Limnol. Oceanogr. 57, 1331–1346. doi:10.4319/lo.2012.57.5.1331

Desloover, J., De Clippeleir, H., Boeckx, P., Du Laing, G., Colsen, J., Verstraete, W., Vlaeminck, S.E., 2011. Floc-based sequential partial nitritation and anammox at full scale with contrasting N_2O emissions. Water Res. 45, 2811–2821. doi:10.1016/j.watres.2011.02.028

Desloover, J., Vlaeminck, S.E., Clauwaert, P., Verstraete, W., Boon, N., 2012. Strategies to mitigate N_2O emissions from biological nitrogen removal systems. Curr. Opin. Biotechnol. 23, 474–482. doi:10.1016/J.COPBIO.2011.12.030

Domingo-Félez, C., Calderó-Pascual, M., Sin, G., Plósz, B.G., Smets, B.F., 2017a. Calibration of the comprehensive NDHA-N 2 O dynamics model for nitrifier-enriched biomass using targeted respirometric assays. Water Res. 126, 29–39. doi:10.1016/j.watres.2017.09.013

Domingo-Félez, C., Mutlu, A.G., Jensen, M.M., Smets, B.F., 2014. Aeration strategies to mitigate nitrous oxide emissions from single-stage nitritation/anammox reactors. Environ. Sci. Technol. 48, 8679–8687. doi:10.1021/es501819n

Domingo-Félez, C., Pellicer-Nàcher, C., Petersen, M.S., Jensen, M.M., Plósz, B.G., Smets, B.F., 2017b. Heterotrophs are key contributors to nitrous oxide production in activated sludge under low C-to-N ratios during nitrification-Batch experiments and modeling. Biotechnol. Bioeng. 114, 132–140. doi:10.1002/bit.26062

Döring, C., Gehlen, H., 1961. Über die Kinetik der Reaktion zwischen Hydroxylamin und Salpetriger Säure. J. Inorg. Gen. Chem. 312, 32–44. doi:10.1002/zaac.19613120106

EPA, 1993. Nitrogen control. US EPA, Washington (DC).

Füssel, J., Lam, P., Lavik, G., Jensen, M.M., Holtappels, M., Günter, M., Kuypers, M.M., 2012. Nitrite oxidation in the Namibian oxygen minimum zone. ISME J. 6, 1200–1209. doi:10.1038/ismej.2011.178

Fux, C., Huang, D., Monti, A., Siegrist, H., 2004. Difficulties in maintaining long-term partial nitritation of ammonium-rich sludge digester liquids in a moving-bed biofilm reactor (MBBR). Water Sci. Technol. 49, 53–60. doi:10.2166/wst.2004.0803

Fux, C., Velten, S., Carozzi, V., Solley, D., Rg Keller, J., 2006. Efficient and stable nitritation and denitritation of ammonium-rich sludge dewatering liquor using an SBR

with continuous loading. doi:10.1016/j.watres.2006.05.003

Gao, K., Zhao, J., Ge, G., Ding, X., Wang, S., Li, X., Yu, Y., 2016. Effect of Ammonium Concentration on N_2O Emission During Autotrophic Nitritation Under Oxygen-Limited Conditions. Environ. Eng. Sci. 00, 1–7. doi:10.1089/ees.2016.0143

Graf, D.R.H., Jones, C.M., Hallin, S., 2014. Intergenomic Comparisons Highlight Modularity of the Denitrification Pathway and Underpin the Importance of Community Structure for N_2O Emissions. PLoS One 9, e114118. doi:10.1371/journal.pone.0114118

Hallin, S., Philippot, L., Löffler, F.E., Sanford, R.A., Jones, C.M., 2018. Genomics and Ecology of Novel N 2 O-Reducing Microorganisms. Trends Microbiol. 26, 43–55. doi:10.1016/j.tim.2017.07.003

Hanaki, K., Hong, Z., Matsuo, T., 1992. Production of Nitrous Oxide Gas during Denitrification of Wastewater. Water Sci. Technol. 26, 1027–1036. doi:10.2166/wst.1992.0544

Harper, W.F., Takeuchi, Y., Riya, S., Hosomi, M., Terada, A., 2015. Novel abiotic reactions increase nitrous oxide production during partial nitrification: Modeling and experiments. Chem. Eng. J. doi:10.1016/j.cej.2015.06.109

Heil, J., Vereecken, H., Brüggemann, N., 2016. A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil. Eur. J. Soil Sci. 67, 23–39. doi:10.1111/ejss.12306

Hellinga, C., Schellen, A.A.J.C., Mulder, J.W., Van Loosdrecht, M.C.M., Heijnen, J.J., 1998. The SHARON process: An innovative method for nitrogen removal from ammonium-rich waste water. Water Sci. Technol. 37, 135–142. doi:10.1016/S0273-1223(98)00281-9

Hiatt, W.C., Grady, C.P.L., 2008. An Updated Process Model for Carbon Oxidation, Nitrification, and Denitrification. Water Environ. Res. 80, 2145–2156. doi:10.2175/106143008X304776

Hughes, M.N., Stedman, G., 1963. Kinetics and mechanism of the reaction between nitrous acid and hydroxylamine. Part I. J. Chem. Soc. 2824. doi:10.1039/jr9630002824

Hussain, M.A., Stedman, G., Hughes, M.N., 1968. Kinetics and mechanism of the reaction between nitrous acid and hydroxylamine. Part III. The formation of hyponitrous acid. J. Chem. Soc. B Phys. Org. 597. doi:10.1039/j29680000597

Hynes, R.K., Knowles, R., 1984. Production of nitrous oxide by Nitrosomonas europaea : effects of acetylene, pH, and oxygen. Can. J. Microbiol. 30, 1397–1404. doi:10.1139/m84-222

Illanes, A., Altamirano, C., Wilson, L., 2008. Homogeneous Enzyme Kinetics, in: Enzyme Biocatalysis. Springer Netherlands, Dordrecht, pp. 107–153. doi:10.1007/978-1-4020-8361-7_3

IPCC, 2013. Climate Change 2013: The Physical Science Basis, Cambridge University Press. Cambridge, United Kingdom and New York, NY, USA. doi:10.1017/CBO9781107415324

Itokawa, H., Hanaki, K., Matsuo, T., 2001. Nitrous oxide production in high-loading biological nitrogen removal process under low COD/N ratio condition. Water Res. 35, 657–664. doi:10.1016/S0043-1354(00)00309-2

Jetten, M.S.M., Horn, S.J., van Loosdrecht, M.C.M., 1997. Towards a more sustainable municipal wastewater treatment system. Water Sci. Technol. 35, 171–180. doi:10.1016/S0273-1223(97)00195-9

Jones, C.M., Hallin, S., 2010. Ecological and evolutionary factors underlying global and local assembly of denitrifier communities. ISME J. 4, 633–641. doi:10.1038/ismej.2009.152

Kampschreur, M.J., Kleerebezem, R., de Vet, W.W.J.M., van Loosdrecht, M.C.M., 2011. Reduced iron induced nitric oxide and nitrous oxide emission. Water Res. 45, 5945–5952. doi:10.1016/j.watres.2011.08.056

Kampschreur, M.J., Tan, N.C.G., Kleerebezem, R., Picioreanu, C., Jetten, M.S.M., Van Loosdrecht, M.C.M., 2008. Effect of dynamic process conditions on nitrogen oxides emission from a nitrifying culture. Environ. Sci. Technol. 42, 429–435. doi:10.1021/es071667p

Kampschreur, M.J., Temmink, H., Kleerebezem, R., Jetten, M.S.M., van Loosdrecht, M.C.M., 2009. Nitrous oxide emission during wastewater treatment. Water Res. 43, 4093–4103. doi:10.1016/j.watres.2009.03.001

Kartal, B., de Almeida, N.M., Maalcke, W.J., Op den Camp, H.J.M., Jetten, M.S.M., Keltjens, J.T., 2013. How to make a living from anaerobic ammonium oxidation. FEMS Microbiol. Rev. 37, 428–461. doi:10.1111/1574-6976.12014

Kartal, B., Kuenen, J.G., van Loosdrecht, M.C.M., 2010. Sewage Treatment with Anammox. Science (80-.). 328, 702–703. doi:10.1126/science.1185941

Kartal, B., Kuypers, M.M.M., Lavik, G., Schalk, J., Op den Camp, H.J.M., Jetten, M.S.M., Strous, M., 2007. Anammox bacteria disguised as denitrifiers: nitrate reduction to dinitrogen gas via nitrite and ammonium. Environ. Microbiol. 9, 635–642. doi:10.1111/j.1462-2920.2006.01183.x

Kartal, B., Maalcke, W.J., de Almeida, N.M., Cirpus, I., Gloerich, J., Geerts, W., Op den Camp, H.J.M., Harhangi, H.R., Janssen-Megens, E.M., Francoijs, K.-J., Stunnenberg, H.G., Keltjens, J.T., Jetten, M.S.M., Strous, M., 2011. Molecular mechanism of anaerobic ammonium oxidation. Nature 479, 127–130. doi:10.1038/nature10453

Khin, T., Annachhatre, A.P., 2004. Novel microbial nitrogen removal processes. Biotechnol. Adv. 22, 519–532. doi:10.1016/J.BIOTECHADV.2004.04.003

Kim, S.W., Miyahara, M., Fushinobu, S., Wakagi, T., Shoun, H., 2010. Nitrous oxide emission from nitrifying activated sludge dependent on denitrification by ammonia-oxidizing bacteria. Bioresour. Technol. 101, 3958–3963. doi:10.1016/j.biortech.2010.01.030

Kinh, C.T., Ahn, J., Suenaga, T., Sittivorakulpong, N., Noophan, P., Hori, T., Riya, S., Hosomi, M., Terada, A., 2017. Free nitrous acid and pH determine the predominant ammonia-oxidizing bacteria and amount of N_2O in a partial nitrifying reactor. Appl. Microbiol. Biotechnol. doi:10.1007/s00253-016-7961-2

Kong, Q., Liang, S., Zhang, J., Xie, H., Miao, M., Tian, L., 2013. N_2O emission in a partial nitrification system: Dynamic emission characteristics and the ammonium-oxidizing bacteria community. Bioresour. Technol. 127, 400–406. doi:10.1016/j.biortech.2012.10.011

Kostera, J., McGarry, J., Pacheco, A.A., 2010. Enzymatic Interconversion of Ammonia and

Nitrite: The Right Tool for the Job. Biochemistry 49, 8546–8553. doi:10.1021/bi1006783

Kowalchuk, G.A., Stephen, J.R., 2001. Ammonia-Oxidizing Bacteria: A Model for Molecular Microbial Ecology. Annu. Rev. Microbiol. 55, 485–529. doi:10.1146/annurev.micro.55.1.485

Law, Y., Lant, P., Yuan, Z., 2013. The Confounding Effect of Nitrite on N 2 O Production by an Enriched Ammonia-Oxidizing Culture. Environ. Sci. Technol. 47, 7186–7194. doi:10.1021/es4009689

Law, Y., Lant, P., Yuan, Z., 2011. The effect of pH on N_2O production under aerobic conditions in a partial nitritation system. Water Res. 45, 5934–5944. doi:10.1016/j.watres.2011.08.055

Law, Y., Ye, L., Pan, Y., Yuan, Z., 2012. Nitrous oxide emissions from wastewater treatment processes. Philos. Trans. R. Soc. B Biol. Sci. 367, 1265–1277. doi:10.1098/rstb.2011.0317

Lemaire, R., Marcelino, M., Yuan, Z., 2008. Achieving the nitrite pathway using aeration phase length control and step-feed in an SBR removing nutrients from abattoir wastewater. Biotechnol. Bioeng. 100, 1228–1236. doi:10.1002/bit.21844

Lin, Y., Guo, M., Shah, N., Stuckey, D.C., 2016. Economic and environmental evaluation of nitrogen removal and recovery methods from wastewater. Bioresour. Technol. 215, 227–238. doi:10.1016/J.BIORTECH.2016.03.064

Liu, G., Wang, J., 2014. Role of Solids Retention Time on Complete Nitrification: Mechanistic Understanding and Modeling. J. Environ. Eng. 140, 48–56. doi:10.1061/(ASCE)EE.1943-7870.0000779

Liu, G., Wang, J., 2013. Long-Term Low DO Enriches and Shifts Nitrifier Community in Activated Sludge. Environ. Sci. Technol. 47, 5109–5117. doi:10.1021/es304647y

Liu, S., Han, P., Hink, L., Prosser, J.I., Wagner, M., Brüggemann, N., 2017. Abiotic Conversion of Extracellular NH 2 OH Contributes to N 2 O Emission during Ammonia Oxidation. Environ. Sci. Technol. 51, 13122–13132. doi:10.1021/acs.est.7b02360

Lu, H., Chandran, K., 2010. Factors promoting emissions of nitrous oxide and nitric oxide from denitrifying sequencing batch reactors operated with methanol and ethanol as electron donors. Biotechnol. Bioeng. 106, n/a-n/a. doi:10.1002/bit.22704

Lu, H., Chandran, K., Stensel, D., 2014. Microbial ecology of denitrification in biological wastewater treatment. Water Res. 64, 237–254. doi:10.1016/j.watres.2014.06.042

Lv, Y., Ju, K., Wang, L., Chen, X., Miao, R., Zhang, X., 2016. Effect of pH on nitrous oxide production and emissions from a partial nitritation reactor under oxygen-limited conditions. Process Biochem. 51, 765–771. doi:10.1016/j.procbio.2016.02.017

Ma, Y., He, X., Meng, L., Xue, X., Zhang, C., 2017. Ionization and separation as a strategy for significantly enhancing the thermal stability of an instable system: a case for hydroxylamine-based salts relative to that for pure hydroxylamine. Phys. Chem. Chem. Phys. 19, 30933–30944. doi:10.1039/C7CP03801A

Mampaey, K.E., De Kreuk, M.K., van Dongen, U.G.J.M., van Loosdrecht, M.C.M., Volcke, E.I.P., 2016. Identifying $_{N2}O$ formation and emissions from a full-scale partial nitritation reactor. Water Res. 88, 575–585. doi:10.1016/j.watres.2015.10.047

Mavrovas, I., 2014. “GraNiti SBR” Start-up and Operation of a Granular Nitritatiing Sequencing Batch Reactor. Technical University of Denmark.

McIlvin, M.R., Altabet, M.A., 2005. Chemical conversion of nitrate and nitrite to nitrous oxide for nitrogen and oxygen isotopic analysis in freshwater and seawater. Anal Chem 77, 5589–5595. doi:10.1021/ac050528s

Monteith, H.D., Sahely, H.R., MacLean, H.L., Bagley, D.M., 2005. A Rational Procedure for Estimation of Greenhouse-Gas Emissions from Municipal Wastewater Treatment Plants. Water Environ. Res. 77, 390–403. doi:10.2175/106143005X51978

Morgan, T.D.B., Stedman, G., Hughes, M.N., 1968. Kinetics and mechanism of the reaction between nitrous acid and hydroxylamine. Part II. The alkyl hydroxylamines. J. Chem. Soc. B Phys. Org. 0, 344. doi:10.1039/j29680000344

Mutlu, A.G., 2015. Management of microbial community composition, architecture and performance in autotrophic nitrogen removing bioreactors through aeration regimes. Technical University of Denmark.

Ni, B.-J., Peng, L., Law, Y., Guo, J., Yuan, Z., 2014. Modeling of Nitrous Oxide Production by Autotrophic Ammonia-Oxidizing Bacteria with Multiple Production Pathways. Environ. Sci. Technol. 48, 3916–3924. doi:10.1021/es405592h

Nielsen, L., 1992. Denitrification in sediment deermined from nitrogen isotope pairing technique. FEMS Microbiol. Lett. 86, 357–362.

Niftrik, L.A., Fuerst, J.A., Damsté, J.S.S., Kuenen, J.G., Jetten, M.S.M., Strous, M., 2004. The anammoxosome: an intracytoplasmic compartment in anammox bacteria. FEMS Microbiol. Lett. 233, 7–13. doi:10.1016/j.femsle.2004.01.044

Pan, Y., Ye, L., Ni, B.-J., Yuan, Z., 2012. Effect of pH on N_2O reduction and accumulation during denitrification by methanol utilizing denitrifiers. Water Res. 46, 4832–4840. doi:10.1016/j.watres.2012.06.003

Park, S., Bae, W., Rittmann, B.E., 2010. Multi-species nitrifying biofilm model (MSNBM) including free ammonia and free nitrous acid inhibition and oxygen limitation. Biotechnol. Bioeng. 105, n/a-n/a. doi:10.1002/bit.22631

Peng, L., Ni, B.-J., Ye, L., Yuan, Z., 2015a. The combined effect of dissolved oxygen and nitrite on N_2O production by ammonia oxidizing bacteria in an enriched nitrifying sludge. Water Res. 73, 29–36. doi:10.1016/j.watres.2015.01.021

Peng, L., Ni, B.-J., Ye, L., Yuan, Z., 2015b. N_2O production by ammonia oxidizing bacteria in an enriched nitrifying sludge linearly depends on inorganic carbon concentration. Water Res. 74, 58–66. doi:10.1016/J.WATRES.2015.02.003

Peng, L., Ni, B.J., Erler, D., Ye, L., Yuan, Z., 2014. The effect of dissolved oxygen on N_2O production by ammonia-oxidizing bacteria in an enriched nitrifying sludge. Water Res. 66, 12–21. doi:10.1016/j.watres.2014.08.009

Philippot, L., Andert, J., Jones, C.M., Bru, D., Hallin, S., 2011. Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N_2O emissions from soil. Glob. Chang. Biol. 17, 1497–1504. doi:10.1111/j.1365-2486.2010.02334.x

Pijuan, M., Torà, J., Rodríguez-Caballero, A., César, E., Carrera, J., Pérez, J., 2014. Effect of process parameters and operational mode on nitrous oxide emissions from a nitritation reactor treating reject wastewater. Water Res. 49, 23–33. doi:10.1016/j.watres.2013.11.009

Poughon, L., Dussap, C.-G., Gros, J.-B., 2001. Energy model and metabolic flux analysis for autotrophic nitrifiers. Biotechnol. Bioeng. 72, 416–433. doi:10.1002/1097-0290(20000220)72:4<416::AID-BIT1004>3.0.CO;2-D

Rathnayake, R.M.L.D., Oshiki, M., Ishii, S., Segawa, T., Satoh, H., Okabe, S., 2015. Effects of dissolved oxygen and pH on nitrous oxide production rates in autotrophic partial nitrification granules. Bioresour. Technol. 197, 15–22. doi:10.1016/j.biortech.2015.08.054

Rodriguez-Caballero, A., Pijuan, M., 2013. N_2O and NO emissions from a partial nitrification sequencing batch reactor: Exploring dynamics, sources and minimization mechanisms. Water Res. 47, 3131–3140. doi:10.1016/j.watres.2013.03.019

Ruiz, G., Jeison, D., Rubilar, O., Ciudad, G., Chamy, R., 2005. Nitrification-denitrification via nitrite accumulation for nitrogen removal from wastewaters. doi:10.1016/j.biortech.2005.02.018

Schreiber, F., Wunderlin, P., Udert, K.M., Wells, G.F., 2012. Nitric oxide and nitrous oxide turnover in natural and engineered microbial communities: Biological pathways, chemical reactions, and novel technologies. Front. Microbiol. 3, 372. doi:10.3389/fmicb.2012.00372

Schulthess, R. v., Kühni, M., Gujer, W., 1995. Release of nitric and nitrous oxides from denitrifying activated sludge. Water Res. 29, 215–226. doi:10.1016/0043-1354(94)E0108-I

Seinfeld, J.H., Pandis, S.N., 2006. Atmospheric chemistry and physics : from air pollution to climate change, 2nd Revise. ed. Wiley-Interscience.

Shaw, L.J., Nicol, G.W., Smith, Z., Fear, J., Prosser, J.I., Baggs, E.M., 2006. Nitrosospira spp. can produce nitrous oxide via a nitrifier denitrification pathway. Environ. Microbiol. 8, 214–222. doi:10.1111/j.1462-2920.2005.00882.x

Shen, L., Guan, Y., Wu, G., 2015. Effect of heterotrophic activities on nitrous oxide emission during nitrification under different aeration rates. Desalin. Water Treat. 55, 821–827. doi:10.1080/19443994.2014.928793

Shiskowski, D.M., Mavinic, D.S., 2006. The influence of nitrite and pH (nitrous acid) on aerobic-phase, autotrophic N_2O generation in a wastewater treatment bioreactor. J. Environ. Eng. Sci. 5, 273–283. doi:10.1139/s05-034

Siegrist, H., Salzgeber, D., Eugster, J., Joss, A., 2008. Anammox brings WWTP closer to energy autarky due to increased biogas production and reduced aeration energy for N-removal. Water Sci. Technol. 57, 383. doi:10.2166/wst.2008.048

Soler-Jofra, A., Picioreanu, C., Yu, R., Chandran, K., van Loosdrecht, M.C.M., Pérez, J., 2018. Importance of hydroxylamine in abiotic N 2 O production during transient anoxia in planktonic axenic Nitrosomonas cultures. Chem. Eng. J. 335, 756–762. doi:10.1016/j.cej.2017.10.141

Soler-Jofra, A., Stevens, B., Hoekstra, M., Picioreanu, C., Sorokin, D., van Loosdrecht, M.C.M., Pérez, J., 2016. Importance of abiotic hydroxylamine conversion on nitrous oxide emissions during nitritation of reject water. Chem. Eng. J. 287, 720–726. doi:10.1016/j.cej.2015.11.073

Stein, L.Y., 2011. Surveying N_2O-Producing Pathways in Bacteria, in: Methods in Enzymology. pp. 131–152. doi:10.1016/B978-0-12-381294-0.00006-7

Strokal, M., Kroeze, C., 2014. Nitrous oxide (N_2O) emissions from human waste in 1970-2050. Curr. Opin. Environ. Sustain. 9–10, 108–121. doi:10.1016/j.cosust.2014.09.008

Strous, M., 2000. Microbiology of anaerobic ammonium oxidation. TU Delft.

Strous, M., Heijnen, J.J., Kuenen, J.G., Jetten, M.S.M., 1998. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. Appl. Microbiol. Biotechnol. 50, 589–596. doi:10.1007/s002530051340

Strous, M., Van Gerven, E., Zheng, P., Kuenen, J.G., Jetten, M.S.M., 1997. Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (Anammox) process in different reactor configurations. Water Res. 31, 1955–1962. doi:10.1016/S0043-1354(97)00055-9

Tallec, G., Garnier, J., Billen, G., Gousailles, M., 2006. Nitrous oxide emissions from secondary activated sludge in nitrifying conditions of urban wastewater treatment plants: Effect of oxygenation level. Water Res. 40, 2972–2980. doi:10.1016/j.watres.2006.05.037

Terada, A., Sugawara, S., Hojo, K., Takeuchi, Y., Riya, S., Harper, W.F., Yamamoto, T., Kuroiwa, M., Isobe, K., Katsuyama, C., Suwa, Y., Koba, K., Hosomi, M., 2017. Hybrid Nitrous Oxide Production from a Partial Nitrifying Bioreactor: Hydroxylamine Interactions with Nitrite. Environ. Sci. Technol. 51, 2748–2756. doi:10.1021/acs.est.6b05521

Terada, A., Sugawara, S., Yamamoto, T., Zhou, S., Koba, K., Hosomi, M., 2013. Physiological characteristics of predominant ammonia-oxidizing bacteria enriched from bioreactors with different influent supply regimes. Biochem. Eng. J. 79, 153–161. doi:10.1016/J.BEJ.2013.07.012

Thörn, M., Sörensson, F., 1996. Variation of nitrous oxide formation in the denitrification basin in a wastewater treatment plant with nitrogen removal. Water Res. 30, 1543–1547. doi:10.1016/0043-1354(95)00327-4

Todt, D., Dörsch, P., 2016. Mechanism leading to N_2O production in wastewater treating biofilm systems. Rev. Environ. Sci. Bio/Technology 15, 355–378. doi:10.1007/s11157-016-9401-2

Tomaszewski, M., Cema, G., Ziembińska-Buczyńska, A., 2017. Influence of temperature and pH on the anammox process: A review and meta-analysis. Chemosphere 182, 203–214. doi:10.1016/J.CHEMOSPHERE.2017.05.003

Turk, O., Mavinic, D.S., 1989. Maintaining nitrite build-up in a system acclimated to free ammonia. Water Res. 23, 1383–1388. doi:10.1016/0043-1354(89)90077-8

Turk, O., Mavinic, D.S., 1986. Preliminary assessment of a shortcut in nitrogen removal from wastewater. Can. J. Civ. Eng. 13, 600–605. doi:10.1139/l86-094

Vadivelu, V.M., Keller, J., Yuan, Z., 2007. Free ammonia and free nitrous acid inhibition on the anabolic and catabolic processes of Nitrosomonas and Nitrobacter. Water Sci. Technol. 56, 89–97. doi:10.2166/wst.2007.612

Vadivelu, V.M., Keller, J., Yuan, Z., 2006. Effect of free ammonia and free nitrous acid concentration on the anabolic and catabolic processes of an enriched Nitrosomonas culture. Biotechnol. Bioeng. 95, 830–839. doi:10.1002/bit.21018

van de Graaf, A.A., Bruijn, P. de, Robertson, L.A., Jetten, M.S.M., Kuenen, J.G., 1996.

Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. Microbiology 142, 2187–2196. doi:10.1099/13500872-142-8-2187

van de Graaf, A.A. van de, Mulder, A., Bruijn, P. de, Jetten, M.S.M., Robertson, L.A., Kuenen, J.G., 1995. Anaerobic oxidation of ammonium is a biologically mediated process. Appl. Environ. Microbiol. 61, 1246–1251.

Van Loosdrecht, M.C.M., Jetten, M.S.M., 1998. Microbiological conversions in nitrogen removal. Water Sci. Technol. 38, 1–7. doi:10.2166/wst.1998.0002

Villaverde, S., Fdz-Polanco, F., García, P.A., 2000. Nitrifying biofilm acclimation to free ammonia in submerged biofilters. Start-up influence. Water Res. 34, 602–610. doi:10.1016/S0043-1354(99)00175-X

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7, 737–750. doi:10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2

Wang, X.-H., Jiang, L.-X., Shi, Y.-J., Gao, M.-M., Yang, S., Wang, S.-G., 2012. Effects of step-feed on granulation processes and nitrogen removal performances of partial nitrifying granules. Bioresour. Technol. 123, 375–381. doi:10.1016/j.biortech.2012.07.080

Warembourg, F.R., 1993. Nitrogen Fixation in Soil and Plant Systems, in: Knowles, R., Henry, B. (Eds.), Nitrogen Isotope Techniques. Academic Press, New York, pp. 127–155.

Wiesmann, U., 1994. Biological nitrogen removal from wastewater. Adv. Biochem. Eng. Biotechnol. 51, 113–154. doi:10.1007/BFb0008736

Wrage, N., Velthof, G.L., Van Beusichem, M.L., Oenema, O., 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biol. Biochem. 33, 1723–1732. doi:10.1016/S0038-0717(01)00096-7

Wunderlin, P., Mohn, J., Joss, A., Emmenegger, L., Siegrist, H., 2012. Mechanisms of N_2O production in biological wastewater treatment under nitrifying and denitrifying conditions. Water Res. 46, 1027–1037. doi:10.1016/j.watres.2011.11.080

Yang, Q., Liu, X., Peng, C., Wang, S., Sun, H., Peng, Y., 2009. N_2O production during nitrogen removal via nitrite from domestic wastewater: Main sources and control method. Environ. Sci. Technol. 43, 9400–9406. doi:10.1021/es9019113

Yang, Q., Peng, Y., Liu, X., Zeng, W., Mino, T., Satoh, H., 2007. Nitrogen Removal via Nitrite from Municipal Wastewater at Low Temperatures using Real-Time Control to Optimize Nitrifying Communities. Environ. Sci. Technol. 41, 8159–8164. doi:10.1021/es070850f

Yang, S., Gao, M.M., Liang, S., Wang, S.G., Wang, X.H., 2013. Effects of step-feed on long-term performances and N_2O emissions of partial nitrifying granules. Bioresour. Technol. 143, 682–685. doi:10.1016/j.biortech.2013.06.066

Yu, R., Chandran, K., 2010. Strategies of Nitrosomonas europaea 19718 to counter low dissolved oxygen and high nitrite concentrations. BMC Microbiol. 10, 70. doi:10.1186/1471-2180-10-70

Yu, R., Kampschreur, M.J., Loosdrecht, M.C.M. van, Chandran, K., 2010. Mechanisms and Specific Directionality of Autotrophic Nitrous Oxide and Nitric Oxide Generation

during Transient Anoxia. Environ. Sci. Technol. 44, 1313–1319. doi:10.1021/es902794a

Yu, R., Perez-Garcia, O., Lu, H., Chandran, K., 2017. Nitrosomonas europaea adaptation to anoxic-oxic cycling: Insights from transcription analysis, proteomics and metabolic network modeling. Sci. Total Environ. doi:10.1016/j.scitotenv.2017.09.142

Zeng, R.J., Lemaire, R., Yuan, Z., Keller, J., 2003. Simultaneous nitrification, denitrification, and phosphorus removal in a lab-scale sequencing batch reactor. Biotechnol. Bioeng. 84, 170–178. doi:10.1002/bit.10744

Zhou, Y., Pijuan, M., Zeng, R.J., Yuan, Z., 2008. Free Nitrous Acid Inhibition on Nitrous Oxide Reduction by a Denitrifying-Enhanced Biological Phosphorus Removal Sludge. Environ. Sci. Technol. 42, 8260–8265. doi:10.1021/es800650j

Zhu-Barker, X., Cavazos, A.R., Ostrom, N.E., Horwath, W.R., Glass, J.B., 2015. The importance of abiotic reactions for nitrous oxide production. Biogeochemistry 126, 251–267. doi:10.1007/s10533-015-0166-4

Zumft, W.G., 1997. Cell biology and molecular basis of denitrification. Microbiol. Mol. Biol. Rev. 61, 533–616.

9 Papers

I. **Su, Q**., Ma, C., Domingo-Félez, C., Kiil, A.S., Thamdrup, B., Jensen, M.M., Smets, B.F., 2017. Low nitrous oxide production through nitrifier-denitrification in intermittent-feed high-rate nitritation reactors. Water Research. 123, 429-438.

II. Blum, J., **Su, Q**.*, Ma, Y., Valverde-Pérez, B., Domingo-Félez, C., Jensen, M., and Smets, B.F., 2018. The pH dependency of N-converting enzymatic processes, pathways and microbes: effect on net-N_2O production. Environmental Microbiology. 20(5), 1623–1640.
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III. **Su, Q**., Domingo-Félez, C., Zhen Z., Blum, J., Jensen, M., and Smets, B.F., N_2O production in an intermittently-fed high-rate nitritation reactor: pH-control is a feasible N_2O mitigation tool. *Submitted to Water Research.*

IV. **Su, Q.**, Domingo-Félez, C., Jensen, M., and Smets, B.F., Abiotic nitrous oxide (N_2O) production shows strong pH dependence, but contributes little to overall N_2O balance in biological nitrogen removal systems. *Submitted to Environmental Science & Technology.*

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